# EFFECT OF VITAMIN B-COMPLEX ON SELECTED CROP PLANTS

DISSERTATION

SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS
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**BOTANY**


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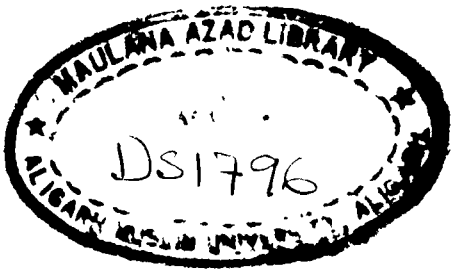
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# I N T R O D U C T I O N

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## INTRODUCTION

Functioning of any organ of an organism is a result of its various physiological changes that in turn are governed by various biochemical reactions. The biochemical reactions, in addition to other substances, require catalysts (enzyme, coenzyme, activator and prosthetic group). The vitamins in general, and B-complex in particular, act as catalyst for various biochemical reactions. If the amount of such a vitamin is not sufficient, the entire system leading to the function of an organ will be affected.

Vitamins of B-complex have been shown as limiting factors for the development of isolated roots, embryos and leaves (Bonner and Bonner, 1948). They have also been noted to increase the uptake of mineral nutrients (Rao and Reddy, 1985). Some varieties of crop plants may be deficient in one or more vitamins due to their genetic constitution, resulting in their poor economic yield. However, the yield of such varieties may be increased by the exogenous application of the vitamin. The possible explanation for this increased yield due to application of supplemental vitamin may be traced as mentioned above to its ameliorating effect on the growth of root and foliage of the plant and direct or indirect effect on uptake of the mineral nutrients.

Cereals and oilseeds play a major role in the agricultural economy of India. With the involvement of new farm technology, India has been able to attain self-sufficiency in cereal production. However, due to high priority accorded to cereals, the oil crops have been neglected, resulting in a wide gap between production and consumption. For example, the total oilseed production in 1987-88 was 12.5 million tonnes against the demand of 26.5 million tonnes (Anonymous 1989) and this situation necessitates heavy imports of edible oils every year.

There are seven major oil yielding crops in India. These include: groundnut, mustard, soybean, sunflower, sesamum, safflower and linseed. Mustard occupies the second position next to groundnut and its production is about 3.8 million tonnes. It occupies one-fifth of the area of cultivated land of the country (Anonymous, 1989). Therefore, it is highly desirable to increase its productivity. The first step in this direction would be to evolve its high yielding varieties. The second step would be to increase per hectare productivity.which can be increased by proper management, planting pattern, balanced manuring and judicious treatment with chemicals, including B-vitamins.

At Aligarh, pre-sowing seed treatment or/and foliar spray of pyridoxine (a member of vitamin B-complex) has been employed successfully to augment the productivity and

quality of a number of crops, including cereals and legumes (Afridi et al., 1979, 1985; Ahmad et al., 1981, 1982, 1986 a,b; Ansari and Samiullah, 1984; Ansari et al., 1985; Khan et al., 1987 a,b; Samiullah et al., 1985, 1987). Some preliminary trials have also been conducted on mustard, indicating a positive response to pyridoxine. However, an in-depth study of its effect on the growth, development, yield and quality of mustard varieties has yet to be undertaken. Therefore, mustard has been selected as test crop for the present study. In all, two experiments have been planned. The aims of these experiments are as follows:

1. To select the most efficacious B-vitamins as well as most responding variety on the basis of yield and quality characters.
2. To select the optimum concentration of the most efficacious vitamin, taking the most responding variety on the basis of the data of Experiment 1.

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# REVIEW OF LITERATURE

# REVIEW OF LITERATURE

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# CHAPTER - 2

# REVIEW OF LITERATURE

## 2.1 Vitamins - an introduction

It was previously believed that food generally consisted of carbohydrates, fats, proteins, salts and water. However, Forster in 1873 observed that pigeons and dogs died within a short period of time when they were fed only the above mentioned food materials. Further, Lunin in 1881 experimenting with mice, found that the addition of fresh milk to the diet kept the experimental animals alive. Thus, he concluded that natural substances, such as milk contain, besides known principles, small quantities of unknown substances essential to life (Rosenberg, 1942).

Later, Hopkins in 1906 called these nutritional elments, required in addition to carbohydrates, fats proteins, salts and water, 'accessory factors'. Funk in 1912 attempted to isolate the compound which prevents beriberi (an animal disease resulting from taking the diet lacking in certain essential constituents - the so called 'accessory factors') and concluded that it is chemically an amine. He proposed the generic term 'vitamine' for the 'accessory factors' because these compounds were essential to life (the Latin term 'vita' meaning life). Finally, Drummond in 1920 dropped the terminal 'e' of 'vitamine' and proposed the generic term 'vitamin' because many of the

compounds of this group were not amines (Rosenberg, 1942).

Folkers in 1969 defined vitamin as "an organic substance of nutritional nature present in low concentration as a natural component of enzyme systems and catalyses required reactions and may be derived externally to the tissues or by intrinsic biosynthesis" (Morton 1974).

Researches after the middle of 1920's recognised various vitamins. Vitamins are divided into fat-soluble vitamin A, D, E and K and water-soluble vitamin B and C (Schopfer, 1949).

It is to be noted that only plants are capable of synthesising vitamins and animals depend directly or indirectly upon plants for their vitamin requirement as supplementary diatry compounds. They are required in very small amounts. The amount of any one vitamin is found less than 5 µg/g of dry weight (Green, 1941).

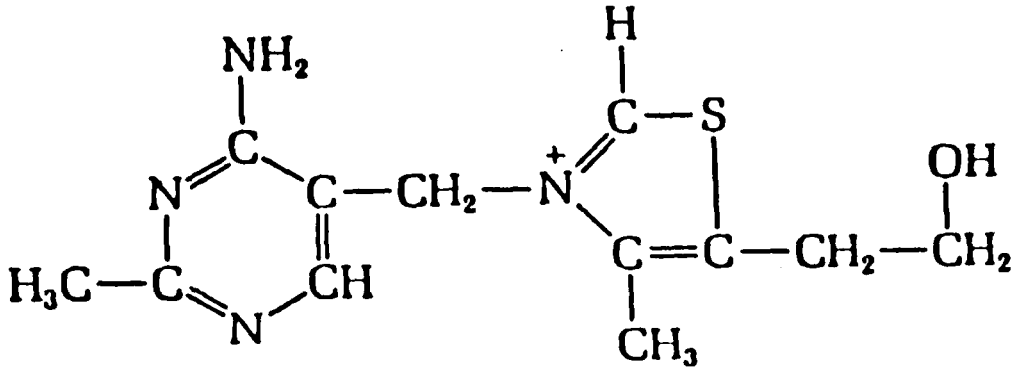
## 2.2 Vitamin B-complex

Osborne and Mendel (1915) and McCollum and Davis (1915) distinguished two types of 'accessory factors' based on difference in their solubility and called them 'fat-soluble A' and 'water-soluble B'. A deficiency of 'water-soluble B' produced beriberi in pigeons. Drummond in 1920 called the water-soluble antiberiberi compound'vitamin B'. What was originally called vitamin B proved to be a mixture of compounds which is referred today as the vitamin B-complex. The vitamins isolated and identified from this

complex were designated as thiamine (vitamin $B_1$), riboflavin (vitamin $B_2$), niacin, pyridoxine (vitamin $B_6$), pantothenic acid, folic acid, cyanocobalamine (vitamin $B_{12}$), biotin, paraminobenzoic acid, inositol and choline. The roles of these vitamins (except paraminobenzoic acid, inositol and choline) in biochemical reactions are well established. In the following pages, their nature, properties and function as components of specific coenzymes would be taken into consideration.

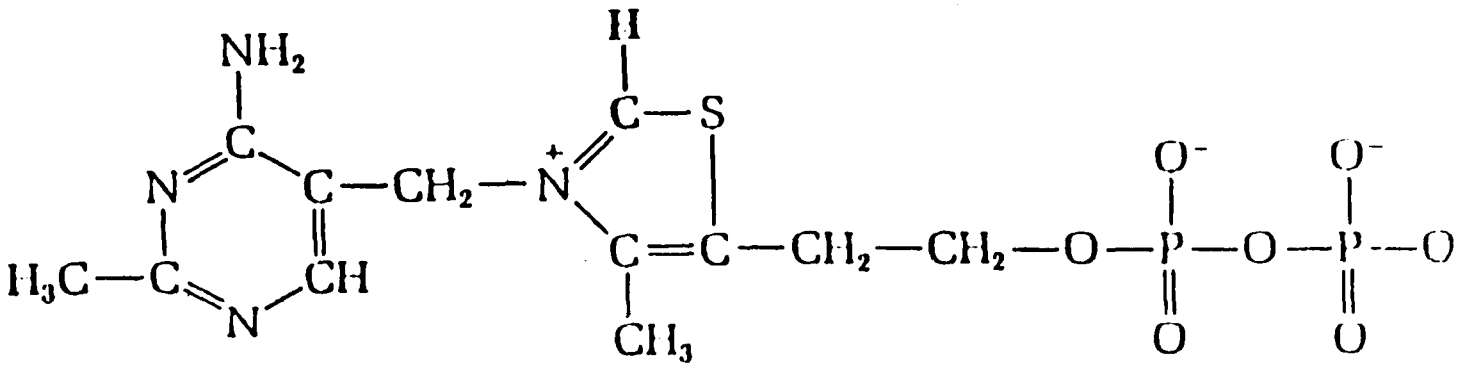
2.2.1 Thiamine

It was first isolated from rice bran by Jansen and Donath (1927) and crystallised by Seidell and Smith (1933). Later, Williams (1936) elucidated its chemical structure and put the empirical formula '$C_{12}H_{18}ON_4SCl_2$'. The molecule of thiamine consists of two units (a) pyrimidine and (b) thiazole in addition to quaternary nitrogen. Both units are joined by a methylene bridge. Structural formula of the molecule is given below:




Thiamine (vitamin $B_1$)

Its biologically active form is thiamine pyrophosphate, which is involved in decarboxylation of α-keto acids. The structural formula is given below:

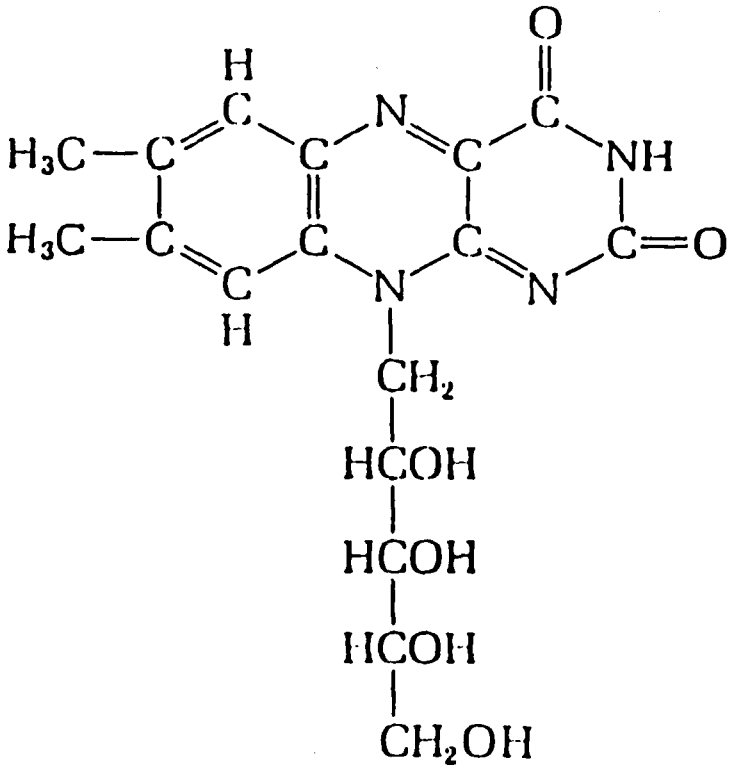


Thiamine pyrophosphate

Regarding to the physical properties of thiamine, it is fairly resistant to boiling but can be destroyed by heating to 120°C for 6 h. It is much soluble in water and alcohols. But insoluble in ether, chloroform, benzene and acetone. Vitamin $B_1$ hydrochloride (salt) crystallises from alcoholic aqueous solutions as the hemihydrate (colourless monoclinic needles) melting at 248 - 250°C (Rosenberg, 1942).

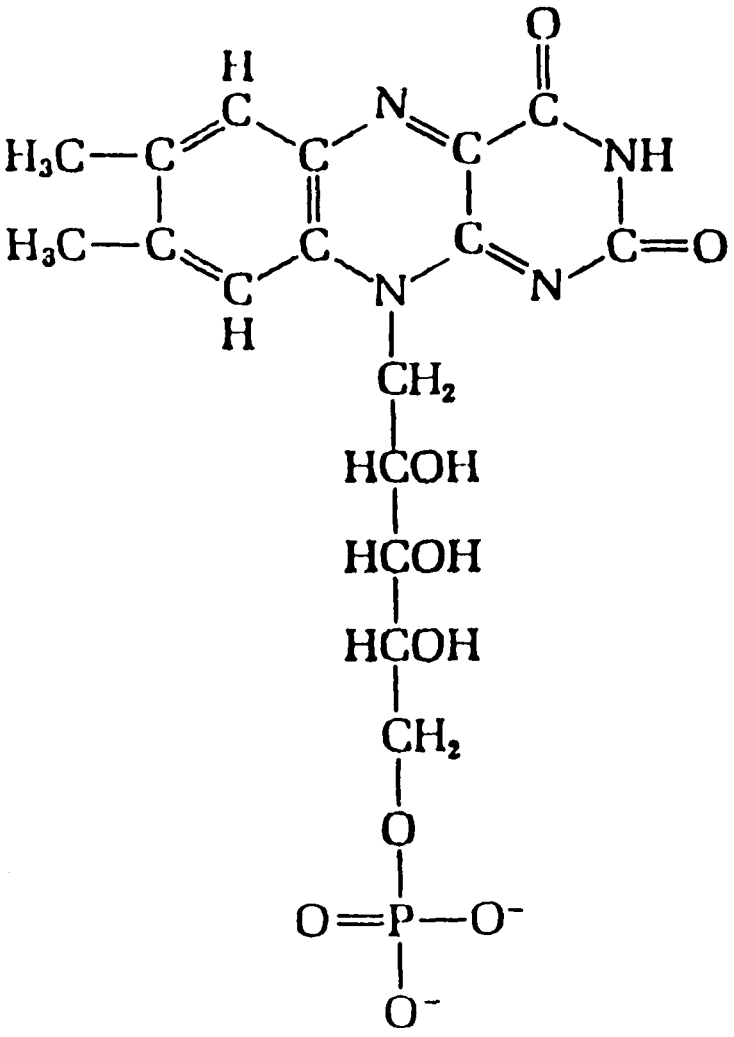
2.2.2 Riboflavin

It was isolated for the first time from whey as an impure flavin by Blyth (1879). Ellinger and Koschara (1933) and Kuhn et al. (1933) isolated pure riboflavin. Later, Kuhn and Karrer and their group established the constitution of riboflavin by total synthesis. It was isolated in crystalline form by Györgyi and Jauregy in 1933. They put forward the empirical formula '$C_{17}H_{20}N_4O_6$' (Rosenberg, 1942). The molecular formula of riboflavin is given below:

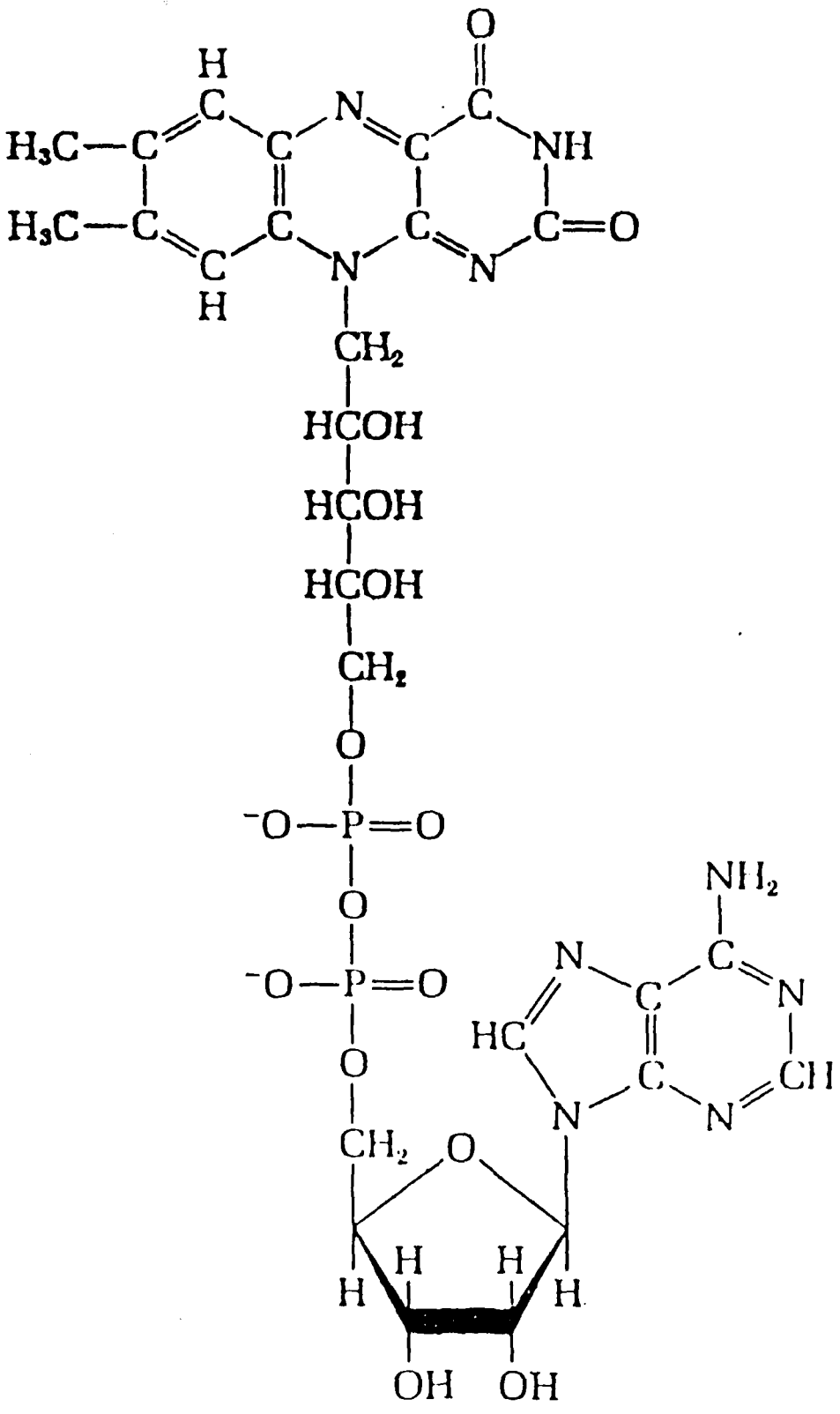


Riboflavin

Like thiamine, riboflavin is biologically active in the form of phosphate, in particular flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD). These are involved in oxidation - reduction reactions. The structural formulae of FMN and FAD are given below:



Flavin mononucleotide (FMN)

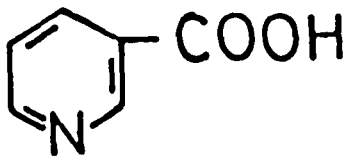


Flavin adenine dinucleotide (FAD)

With regard to its physical properties, pure riboflavin consists of fine orange yellow crystals. It possesses a very bitter taste having melting point about 280°C under decomposition. Crystalline riboflavin is stable under ordinary storage conditions, although it is advisable to protect it from light. Pure riboflavin is soluble in water and in ethyl alcohol, amyl alcohol, phenol, amyl acetate, etc. It is insoluble in acetone, ether, chloroform and benzene (Rosenberg, 1942).

2.2.3 Niacin

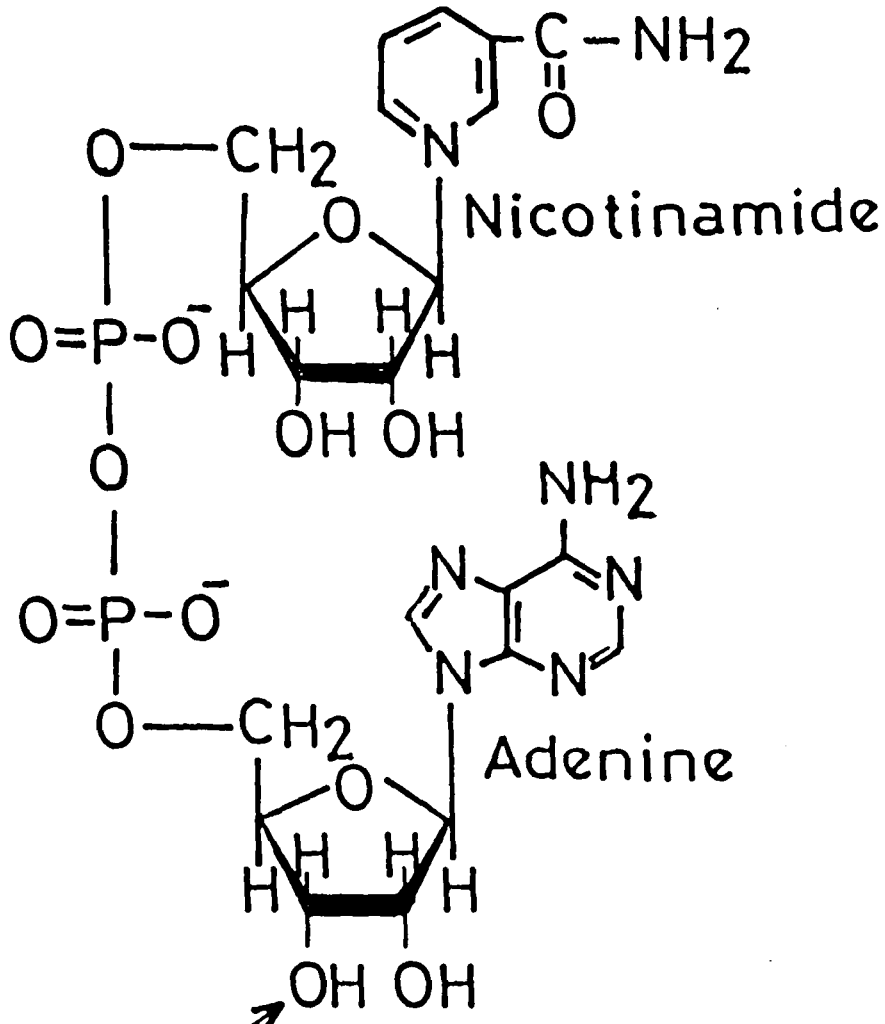
It is known as nicotinic acid and was isolated from yeast and rice bran by Suzuki *et al.* (1912) and Funk (1913a,b) They put forward the empirical formula '$C_6H_5O_2N$'. The structural formula of nicotinic acid is given below:




Nicotinic acid
(niacin)

The biologically active form of nicotinic acid is its amide, which constitutes a part of nicotinamide adenine dinucleotide (NAD) and nicotinamide adenine dinucleotide

phosphate (NADP). These coenzymes are involved in oxidation-reduction reactions. The structural formulae of NAD and NADP are given below:



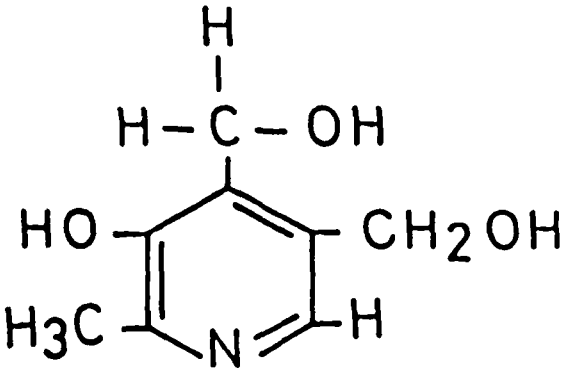

In NADP this hydroxyl group is esterified with phosphate

Nicotinamide adenine dinucleotide ($NAD^+$)

With regard to its physical properties, nicotinic acid crystallises in needles from water or alcohol and melts at 235.5-236.5°C. It sublimes without decomposition (Rosenberg, 1942).

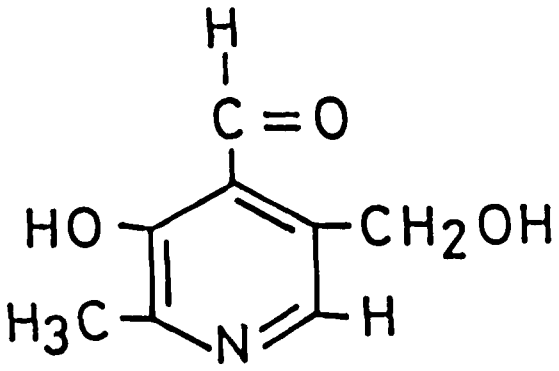
2.2.4 Pyridoxine

It was isolated from rice polishings as early as 1932 by Ohdake in Japan. However, its properties were not recognised. After six years, in 1938, the vitamin was isolated in the pure crystalline form by five different groups (Rosenberg, 1942). György and Eckhardt (1939) suggested the term pyridoxine. Kuhn and Wendt (1938, 1939 a,b), Keresztesy and Stevens (1938) and Harris et al. (1939), working independently, determined the chemical structure of the substance. The vitamin has the empirical formula $C_8H_{11}O_3N$. The structural formula of the vitamin is given below:

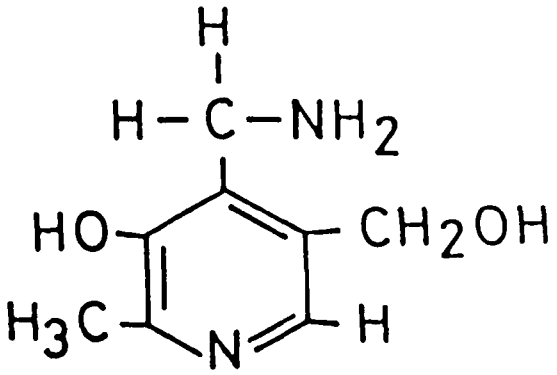



Pyridoxine

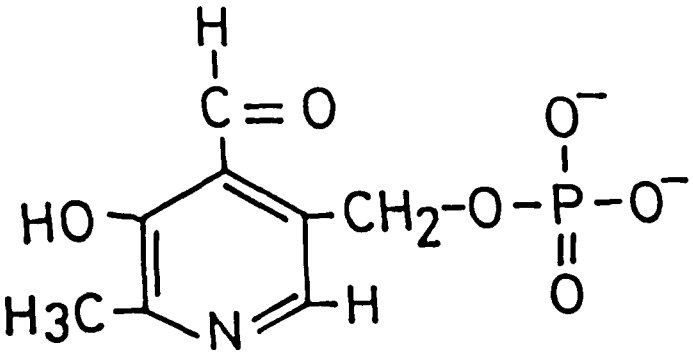
Pyridoxine, pyridoxal and pyridoxamine constitute vitamin $B_6$ group. The active form of vitamin $B_6$ is pyridoxal phosphate which also occurs in its amino form pyridoxamine phosphate. Pyridoxal phosphate serves as prosthetic group, involving in amino group transfer reactions. The structural formulae of pyridoxal, pyridoxamine and pyridoxal phosphate are given below:



Pyridoxal



Pyridoxamine

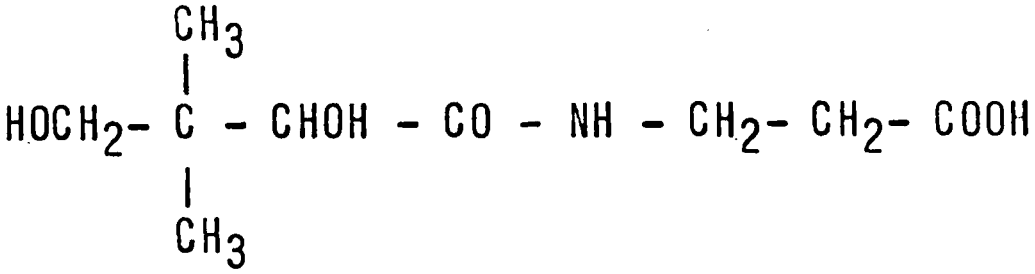


Pyridoxal phosphate

Pyridoxine, as a free base, is a colourless crystalline powder, has a slightly bitter taste and melts at 160°C (Itiba and Miti, 1938; Kresztesy and Stevens, 1938). It is readily soluble in water, alcohol and acetone and slightly soluble in ether and chloroform. It forms salts easily with acids such as hydrochloric acid, picric acid, etc. It is commercially available as its heat stable white hydrochloride salt, with melting point 204-206°C (with decomposition). The hydrochloride salt is soluble in water and in alcohol.

2.2.5 Pantothenic acid

It was first isolated in 1938 from yeast and liver extracts by Williams (Lehninger, 1982). The chemical nature of pantothenic acid was worked out by Williams *et al.* (1939). It is a component of coenzyme A, which is involved in acyl-group transfer. Its empirical formula is $C_9H_{17}O_5N$. The structural formula of the vitamin is given below:

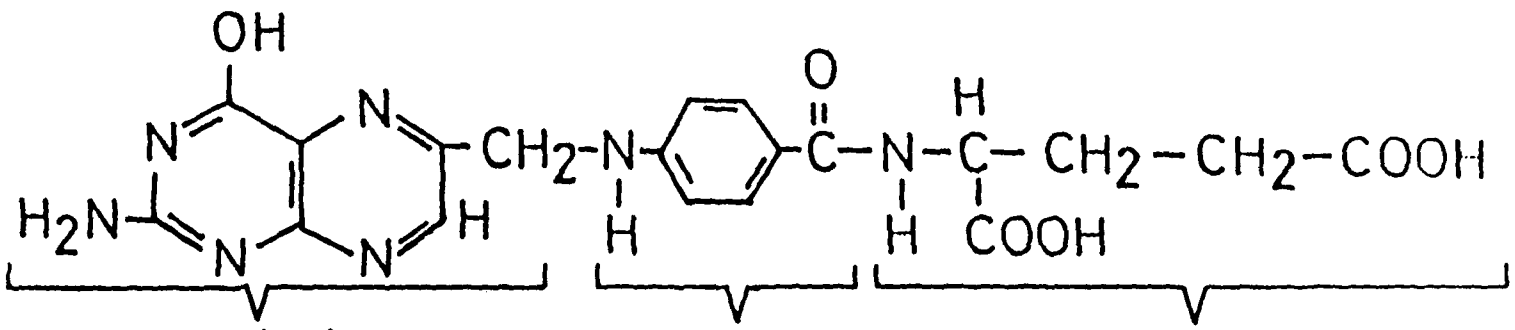


Pantothenic acid

Pantothenic acid is predominantly of acidic character but also shows some basic properties. The vitamin is readily soluble in water, ethyl acetate, dioxane, glacial acetic acid, etc. At room temperature, it has the consistency of a yellowish oil. Its sodium salt, however, crystallises as colourless, very hydroscopic needles. It occurs as a fine, white, odourless, crystalline powder possesing a slightly bitter taste.

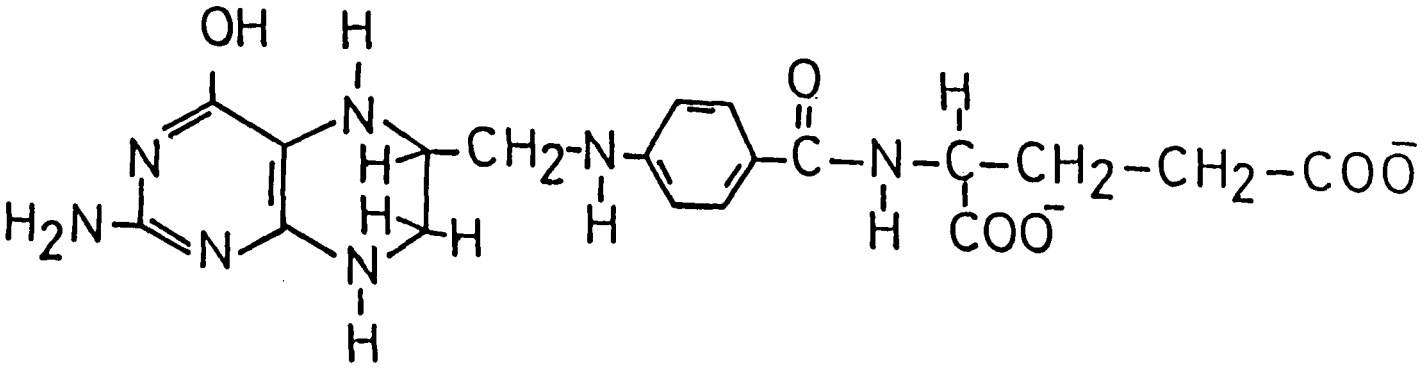
2.2.6 Folic acid

It was first isolated from spinach leaves by Mitchell et al. (1941). It has three major components: glutamic acid, p-amino benzoic acid and pteroylglutamic acid. Mowat et al. (1946) established the molecular structure of the vitamin. The structural formula of the vitamin is given below:




Folic acid

Folic acid has no conenzyme activity itself, but it is enzymatically reduced in the twines to tetrahydrofolic acid ($FH_4$), its active coenzyme form. Tetrahydrofolate functions as an intermediate carrier of 1 carbon groups in a number of complex enzymatic reactions. The structural formula of tetrahydrofolic acid is given below:

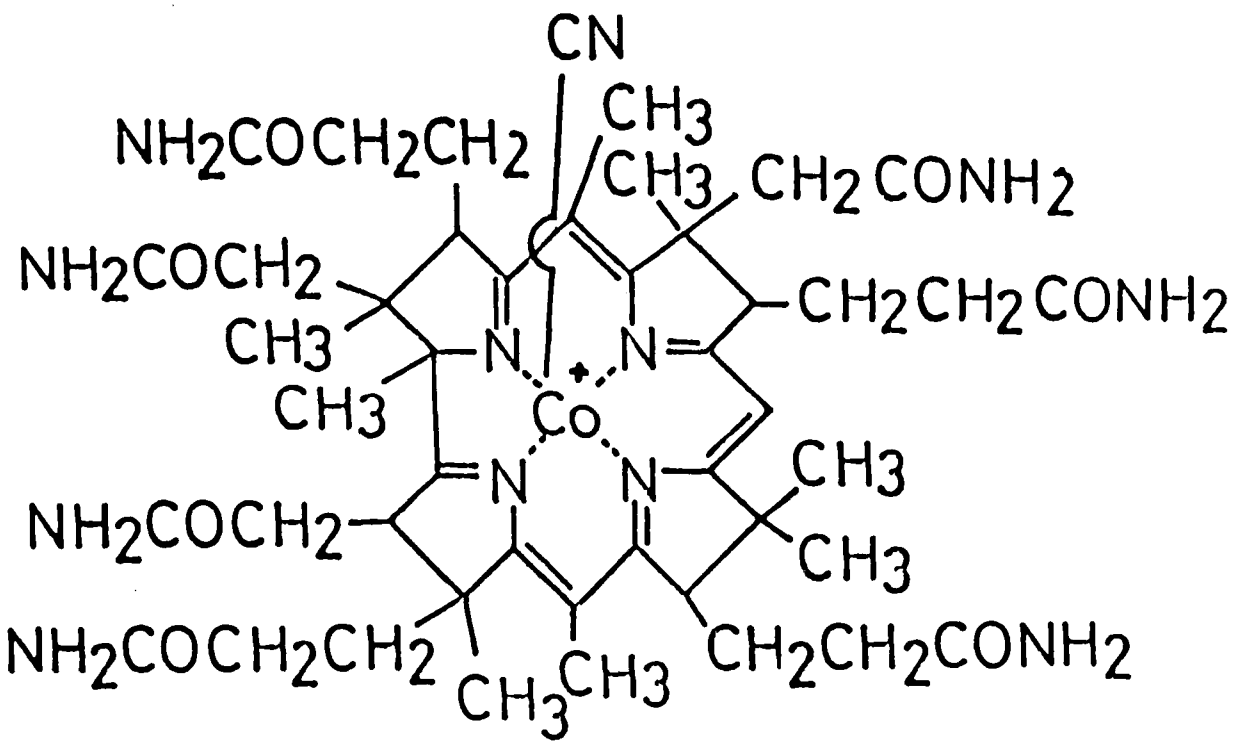


Tetrahydrofolate

Folic acid of commerce is a yellow, crystalline powder, insoluble in water, alcohol, chloroform, ether or benzene. It is sparingly soluble in hot water.

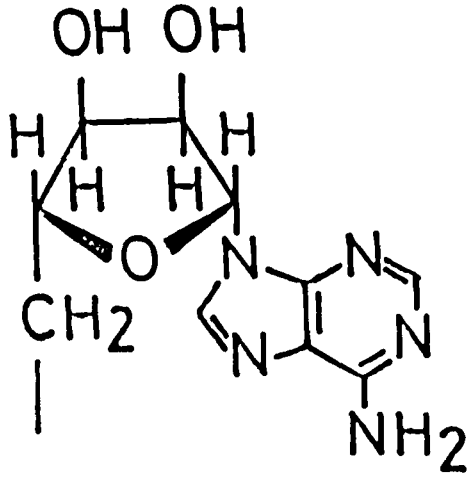
2.2.7 Cyanocobalamine

Rickes et al. (1948) isolated it in crystalline form from liver extracts. In a restricted sense vitamin $B_{12}$, as usually isolated, is called cyanocobalamine. Vitamin $B_{12}$ in a wide sense usually means the whole group of substances, all of which possess a porphyrin like cyclic structure around a cobalt atom. Hodgkin et al. (1955) determined its elemental composition as $C_{63}H_{90}O_{14}N_{14}PCO$. The structural formula of the vitamin was given by Fries (1961) and is depicted below:




Cyanocobalamine

Vitamin $B_{12}$ is unique among all the vitamins in that it contains not only a complex organic molecule but also an essential trace element-cobalt. When the cyano group of cyanocobalamine is replaced by the 5-deoxyadenosyl group (structural formula given below),

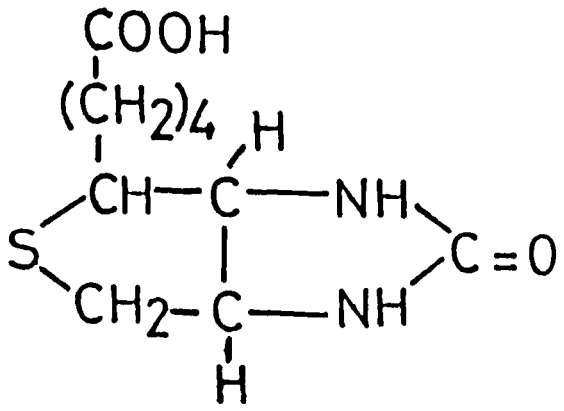



5′-Deoxyadenosyl group of coenzyme $B_{12}$

the vitamin becomes converted to the coenzyme form (also coenzyme $B_{12}$), which is involved in reactions wherein shifting of a hydrogen atom from one carbon atom to the adjacent one or exchange of alkyl, carboxyl, hydroxyl or amino group takes place. The various forms of vitamin $B_{12}$ crystallise as red needles and are not very soluble in water. The water solution has its maximum stability at pH 4.5-5.0.

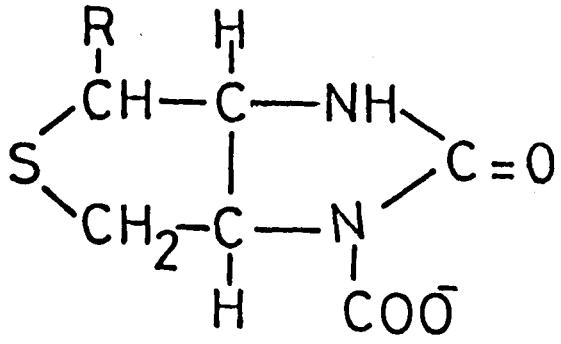
2.2.8 Biotin

Biotin was isolated in crystalline form from dried egg yolks by Kogl (1935). Its empirical formula is $C_{10}H_{16}O_3N_2S$. The structural formula for biotin was suggested by Vigneaud et al. (1942) and is given below:



Biotin

It is a component of biocytin which is involved in $CO_2$ transfer reactions. The structural formula of biocytin is given below:



Biocytin

Where R = $NAD^+$

Biotin is also known as vitamin H. It is water and alcohol soluble, but is relatively insoluble in chloroform, ether and petroleum ether. The vitamin is essentially heat stable, readily dialyzable and resistant to treatment with acid or alkali. The pure vitamin melts at 230-232$^{o}$C. The isoelectric point appears to be between pH 3 and 3.5.

## 2.3 Distribution, transport and excretion of B-vitamins

B-vitamins are found in all species and in all organs of higher plants. They are synthesised in particular restricted areas, like green mature leaves and are subsequently translocated to the growing organs, like shoot apices, root and reproductive organs (Bonner and Bonner, 1948). Some part of the vitamins is also excreted through roots. The excreted vitamins provide suitable medium for the growth of microflora in rhizosphere which are beneficial for the growth of plants (Day, 1943).

## 2.4 Effect of B-vitamins on physiomorphology of crop plants

It is clear from the previous discussion that the vitamins enter into the enzyme systems and act as prosthetic groups and thus control various metabolic activities. The metabolic activities are manifested in the form of growth, development and differentiation. Therefore, the various characteristics of growth and development could be used as

markers for the effect of a vitamin on metabolism. Keeping this view in mind, a number of researches has been conducted to study the effect of exogenously applied B-vitamins on the physiomorphology of plants *in vitro* and *in vivo*. The following review deals with crop plants and is categorised on the basis of modes of application of the vitamin/s, including (1) pre-sowing seed treatment (2) seed or organ treatment in culture medium (3) foliar treatment and (4) combined pre-sowing seed and foliar treatment.

### 2.4.1 Pre-sowing seed treatment

In nature, seeds are well furnished with sufficient quantity of food reserves, minerals, vitamins and hormones for development of embryo until it becomes an independent plant. However, in seeds of some of the newly evolved high yielding varieties of crop plants, the vitamin content remains insufficient or decreases with storage time, resulting in poor germination and growth and development. In such cases, the exogenous application of vitamins as pre-sowing seed treatment may prove effective. In the following paragraphs, the available literature on pre-sowing seed treatment with vitamin/s is reviewed briefly:

Iijima (1952) soaked the seeds of Kentucky Wonder and Masterpiece kidney bean, Golden Bantam maize and Miyashige Japanese radish in solutions of various concentratior

of vitamin $B_1$ for 24 h prior to sowing. Vitamin $B_1$ was used in concentration ranging from 0.0000001 to 100 ppm. The vitamin accelerated the growth of plumules and radicles, especially at an early stage of germination, and also increased the percentage of germination in old seeds. Optimum concentration of vitamin $B_1$ was found to be 0.01 ppm. The application of 0.05 ppm of the vitamin also increased respiration as well as vitamin $B_1$ concentration in cotyledons by 150-180% over the control. Measurement of the amount of both free and ester type of the vitamin in 1 to 4 year old seeds showed that the concentration of both types of the vitamin decreased with the age.

Kjelvik (1965) observed that the soaking of seeds of cucumber, radish and lettuce in nicotinic acid for 2 h resulted in increased yield. Similarly, Aĭzikovick (1967) noted that the treatment of vitamin $B_1$ and $B_2$ to rice seeds resulted in a high rate of field emergence. Plants grown from treated seedsaveraged 9% higher yield than the control plants.

Gŭtmanis (1967) noted that soaking of pea seeds in 0.01% vitamin $B_1$ increased the yield of pea by 15%. Further, seed treatment with the vitamin C, pyridoxal phosphate and vitamin $B_1$ increased the vitamin C content of the crop by an average of 12% and total sugar content by 40%.

Genkel' (1970) studied the effect of pre-sowing seed treatment for 12 h with nicotinic acid and nicotinamide solutions on grain yield and protein content of winter wheat cv. Uljanovka. He soaked the seeds in 0.005 (a), 0.05 (b) and 0.5% (c) nicotinic acid solution and in 0.05 (d) and 0.5% (e) nicotinamide solution. Seed yield increased from 16.7 q/ha in the control to 21.9 by (a), 18.8 by (b) and 24.5 q/ha by (d). However, the seed yield was reduced to 14.3 q/ha by (c). Seeds soaked in (a), (b), (c), (d) and (e) contained 89.2, 100.2, 96.3, 87.9 and 80.3 mg protein/g dry matter respectively compared with 82.7 mg in untreated seeds (control).

Serebryakova (1971) soaked the seeds of Rosa cinnamomea in either 0.01% nicotinic acid or 0.02% vitamin $B_1$ and noted that the soaking advanced germination by 3-4 days and increased emergence by 43 and 51% respectively as compared to the control. She further noted that the treatment with vitamin $B_1$, $B_2$, C or nicotinic acid stimulated growth and synthesis of nicotinic acid and increased plant quality and productivity of hips.

Sinkovics (1974) treated the seeds of two Capsicum cvs. with 7 different vitamins of B group. He concluded that the treatments advanced germination and improved seedling vigour and earliness, size and quality of the crop.

Kulieva *et al.* (1976) studied the reaction of the melon cv. Sary Gulyabi and the water melon cv. Zimnu-344 to treatment with vitamins. The seeds were treated with thiamine, cyanocobalamine, nicotinic acid, pyridoxine or ascorbic acid at concentration ranging from 0.01 to 0.0001%. The effect of the individual treatment was studied on stem and root development at 45d and number and weight of fruit at 90d. The best results were noted when the seeds were treated with 0.001% thiamine or 0.0001% nicotinic acid and 0.0001% cyanocobalamine.

Reda *et al.* (1977) observed that thiamine applied at 50 mg/l by seed soaking for 48 h promoted root, stem, leaf, umbel and fruit growth of *Ammi visnaga*.

Serebryakova and Kalanova (1977) observed that the pre-sowing treatment of seeds of *Rosa cinnamomea* with vitamin $B_1$, $B_2$, or nicotinic acid increased germination by 43, 51, 30 and 30% respectively compared with the control. The treatment increased plant vigour and the output of stand and seedling/ha and improved their quality.

Afridi *et al.* (1979) observed that among the known growth promoting substances, including phytohormones and vitamins, seed treatment with pyridoxine (vitamin $B_6$) gave very promising results in preliminary screening to test their effect on root growth of a number of crop plants of

which barley variety K 572/28 was selected for subsequent detailed studies. They soaked barley seeds in 0.0, 0.1, 0.3, and 0.5% pyridoxine solution for 24 h. These treated seeds were sown in sand culture. After 50 days of sowing, roots and shoots were analysed for their growth characteristics. Yield and quality of grain were studied at harvest. They noted that the treatment of seeds had significant beneficial effect on most of the root, shoot, yield and quality parameters. The two higher concentrations of the vitamin (0.3 and 0.5%) proved best for most of the characteristics studied.

Ahmad et al. (1981), in a factorial randomised block design field experiment, studied the effect of pre-treatment of grain with pyridoxine on the growth of five varieties of barley. They soaked the seed of barley varieties NP-13, NP-21, K 572/10, K 572/28 and Clipper for 24 h in aqueous pyridoxine solution (0.0, 0.02, 0.1 and 0.5%) before sowing. They noted a significant effect of the treatments on tiller number, leaf number, shoot length and fresh and dry weight at tillering, heading and milky grain stages. Seed treatment with 0.1% solution proved optimum at all stages of growth. Tiller and leaf number were maximum in Clipper and minimum in K 572/28 but the latter produced the tallest plants. NP-21 had the maximum fresh and dry weight followed by K 572/28. Treatment x variety interaction effect was mostly significant. The response of various combinations varied

from character to character and stage to stage but the combination 0.02% pyridoxine and K 572/28 proved best.

Ahmad et al. (1982) further pointed out that, in the above trial, maximum grain yield and straw yield were recorded in 0.1% and 0.02% pyridoxine treatment respectively. K 572/28 and NP 21 out yielded all others in grain yield and straw yield respectively. Combination 0.1% X K 572/10 produced maximum grain yield and 0.02% X NP 13, maximum straw yield.

Ashfaq et al. (1983) studied the effect of soaking the grain of triticale var. Branco-90 for 12 h in 0.0, 0.1, 0.2, 0.3 and 0.4% aqueous pyridoxine solution on its germination and yield characteristics. Most of the yield attributes were significantly affected by the treatments. Highest yield (37.7% more than in unsoaked control) was obtained in 0.2% treatment presumably due to higher grain weight and per cent germination.

Ansari and Samiullah (1984) conducted a simple randomised field experiment on lentil (Lens culinaris Medic. var. T-36) to study the effect of pre-sowing seed treatment with pyridoxine on different yield parameters. The seeds were soaked in water, 0.1, 0.2, 0.3, 0.4 and 0.5% aqueous pyridoxine solution for 12 h before sowing, with unsoaked seeds providing the second control. At harvest, pods/plant,

pod length, seeds/pod, 1000 seed weight and seed yield were noted.Pyridoxine significantly affected all the parameters positively, except pod length. Soaking in 0.3% solution proved optimum.

Khan and Ansari (1984) conducted a sand culture experiment on Phaseolus radiatus var. T-9 to find out the effect of pyridoxine on various growth characteristics. Seeds were soaked in 0.0, 0.1, 0.2 and 0.3% aqueous pyridoxine solution for 10 h before sowing. Ten days old seedlings were sampled for growth parameters. Pyridoxine significantly affected the growth parameters, with 0.1% increasing the fresh weight and number of lateral roots.

Khan and Zaidi (1985) conducted a simple randomised field trial with Brassica juncea L. Czern. & Coss. var. Varuna to study the effect of pre-sowing seed treatment with 0.0, 0.05, 0.1 & 0.2% aqueous pyridoxine solution for 4h. Of the treatments, 0.05% proved optimum for height/plant, fresh weight/plant at 50, 70, and 90d, NAR at 70 and 90d growth and pods/plant, seeds/pod, hectolitre weight, seed yield and oil yield at harvest. A significant increase of 13.8 and 16.7% with 0.05% pyridoxine solution was noted in seed and oil yield respectively over the water soaked control.

Samiullah et al. (1985) studied the effect of pre-sowing seed treatment for 4h with 0.0, 0.1, 0.2, 0.3, 0.4

and 0.5% aqueous pyridoxine solution on NRA activity, root length and root nodule number of summer moong (Vigna radiata) var. K-851 at 20, 30, 40 and 50d. Correlations between NRA at the four growth stages and seed yield were also studied. Soaking in 0.3% pyridoxine solution increased NRA by 29.7% at 20d, 7.1% at 30d, 11.8% at 40d and 15.6% at 50d. Root nodule number was maximum in 0.3% at all stages and root length in 0.1% at 20d. Nitrate reductase activity was significantly correlated with seed yield at 20 and 50d. However, there was no significant correlation at 30 or 40d.

Ahmad et al. (1986a) reported that in an earlier trial (Ahmad et al., 1981) the combination 0.01% x K 572/10 proved optimum for ear weight/plant, length/ear, spikelets/ear and grains/ear. Ahmad et al. (1986b) further reported that in the above trial the treatments significantly affected 1000 grain weight, grain carbohydrate and protein percentage among the quality parameters, but the differences were not marked. K 572/28 gave the highest 1000 grain weight, carbohydrate and protein percentage. Treatment X variety interactions were significant for 1000 grain weight and grain protein percentage, but the differences were not marked.

Ansari and Khan (1986) conducted a simple randomised field trial with summer moong var. K-851 to study the effect of pre-sowing seed treatment with 0.0, 0.1, 0.2, 0.3, 0.4 and 0.5% aqueous pyridoxine solution for 4h. The treatments

significantly enhanced plant length, leaf number, fresh and dry weight at 30, 40 and 50; NAR at 20-30, 30-40 and 40-50d growth and pods/plant, pod length, seeds/pod at harvest. Pyridoxine supply at 0.3% was found optimum. Seed yield was positively correlated with plant length at 30 and 50d; with leaf number and dry weight at 30, 40 and 50d; with fresh weight at 30 and 40d and with NAR at all intervals. Seed protein content showed positive correlation with plant length at 30, 40 and 50d; with fresh and dry weight at 40 and 50d and with NAR at 30-40 and 40-50d. Pod number, pod length, and seeds/pod were correlated with seed yield only.

Ansari (1986) studied the effect of pre-sowing seed treatment for 12h with 0.0, 0.1, 0.2, 0.3, 0.4 and 0.5% aqueous pyridoxine solution on growth parameters (plant length, root length, root nodule number, leaf number, fresh weight and dry weight), net assimilation rate (NAR), nitrate reductase activity (NRA), leaf NPK content, yield parameters (pods/plant, pod length, seeds/pod, 1000 seed weight and seed yield) and seed protein content of lentil var. T-36. An unsoaked control was also included in the scheme. The growth parameters, NRA and leaf NPK content were studied at 60, 90 and 120d; NAR was computed for the periods 60-90 and 90-120d and yield parameters and seed protein content were studied at harvest. 0.3% proved optimum for growth parameters

at 90 and 120d; for NAR at both intervals; for NRA and leaf NPK content at all three stages and for yield parameters (except pod length and 1000 seed weight) and seed protein content at harvest.

Khan et al. (1987) conducted a factorial randomised field experiment on mustard (Brassica juncea L. Czern. & Coss.) var. Varuna. They studied the effect of pre-sowing seed treatment for 4h with 0.0125, 0.025, 0.05 and 0.10% aqueous pyridoxine solution and basal $N_{60}P_{20}$, $N_{90}P_{30}$ and $N_{60+30}P_{30}$, alone as well as in combination, on the performance of mustard. The parameters studied included: shoot and root length, fresh weight, leaf NPK at 50, 70 and 90d and net assimilation rate (NAR) for the periods 50-70 and 70-90d after sowing. Two controls (unsoaked and water-soaked) were maintained. Soaking of seeds with 0.025% pyridoxine gave maximum value for all parameters studied, including leaf NPK. 60 kg N and 20 kg P/ha ($N_{60}P_{20}$), applied at sowing, proved best for all parameters, except plant height. The interactions 0.0125 x $N_{60}P_{20}$, 0.025 x $N_{60}P_{20}$, 0.025 x $N_{90}P_{30}$ (being at par) proved optimum for all parameters studied. The data, particularly with respect to leaf NPK content, showed that soaking in pyridoxine solution enhanced growth and facilitated nutrient uptake in mustard.

Samiullah et al. (1987), in a field experiment, studied the effect of pre-sowing seed treatment for 4h with

0.0125, 0.025, 0.05 and 0.10% aqueous pyridoxine solution and three combinations of fertiliser N and P ($N_{60}P_{20}$, $N_{90}P_{30}$ and $N_{60+30}P_{30}$), alone and in combination, on pods/plant seeds/pod, hecto-litre weight, oil percentage, oil and seed yield as well as acid, iodine and saponification values of oil of mustard (Brassica juncea L. Czern. & Coss.) var. Varuna. Unsoaked as well as water-soaked controls were included for comparison. Soaking in 0.025% pyridoxine proved optimum for almost all yield and quality parameters, giving 14.9% more seed yield than the controls, while treatment $N_{60}P_{20}$ gave maximum value for all the parameters, except the hecto-litre weight. Similarly, the interaction 0.0125 x $N_{60}P_{20}$ proved optimum for most parameters, but was at par with 0.025 x $N_{60}P_{20}$ and 0.025 x $N_{90}P_{30}$. The correlation studies showed that leaf NPK, growth and yield parameters were positively correlated with seed yield. It was therefore, inferred that pre-sowing seed treatment with 0.0125% pyridoxine solution favours nutrient uptake, growth and development of mustard and enhances seed yield and resulted in a saving of 30 kg N and 10 kg P/ha to the farmers.

Khan (1988) conducted an experiment on summer moong var. K-851. He studied the effect of four doses of basal nitrogen, i.e. 0, 5, 10, 15 kg N/ha and pre-sowing seed treatment for 4h with aqueous pyridoxine solution, i.e. 0.0 , 0.2, 0.3 and 0.4% pyridoxine, alone as well as in

combination, on growth parameters, NRA and leaf NPK content at 20, 30, 40 and 50d, NAR for 20-30, 30-40 and 40-50d intervals and yield parameters and seed protein content at harvest. Of these, $N_5$ and 0.3% separately proved optimum for almost all parameters. Among different interactions, $N_5$ x 0.2% proved optimum for most of the parameters. In another field experiment on summer moong var. K-851, he studied the effect of basal phosphorus, viz. 15, 30, 45, and 60 and pre-sowing seed treatment for 4h with aqueous pyridoxine solution, i.e. 0.01, 0.2, 0.3, and 0.4%, alone as well as in combination, on growth parameters, NRA and leaf NPK content at 20, 30, 40 and 50d, NAR for 20-30, 30-40 and 40-50d intervals, and yield parameters and seed protein content at harvest. Among these, $P_{30}$ and 0.3% separately proved optimum for all parameters, while $P_{15}$ x 0.3% emerged as the best combination for all parameters. He also studied the effect of basal nitrogen at 15, 30, 45 and 60 kg N/ha and pre-sowing seed treatment for 12h with aqueous pyridoxine solution, i.e. 0.0, 0.2, 0.3 and 0.4% on growth parameters, NRA and leaf NPK content at 60, 90 and 120d, NAR for 60-90 and 90-120d intervals and yield parameters and seed protein content at harvest of lentil var. T-36. Of the nitrogen doses, $N_{30}$ proved optimum for most parameters. The soaking treatment 0.3% proved optimum for almost all parameters studied. The interaction $N_{30}$x 0.3% proved optimum and showed nutrient use efficiency.

Khan et al. (1988) studied the effect of basal phosphorus at 15, 30, 45 and 60 kg P/ha and pre-sowing seed treatment for 12h with aqueous pyridoxine solution, i.e. 0.0, 0.2, 0.3 and 0.4% alone as well as in combination on nitrate reductase activity (NRA) at 60, 90 and 120d. Net assimilation rate (NAR) for 60-90 and 90-120d intervals and pods/plant, pod length, seeds/pod, 1,000 seed weight, seed yield and seed protein content at 140 d (harvest) of lentil var. T-36. Application of 30 kg P/ha and 0.3% pyridoxine separately and interaction 30 kg P/ha x 0.2% proved best for most parameters.

### 2.4.2 Seed or organ treatment in culture medium

Just like pre-sowing seed treatment, seed or organ of a plant can also be treated in culture medium having a sufficient quantity of vitamin/s. The available literature in this regard is reviewed below:

Bonner (1937), working with excised pea roots in culture medium, found that pea roots ceased to grow completely after three transfers in the absence of yeast extract. Addition of 0.01% yeast extract to the nutrient medium had a beneficial effect on the growth of roots with an average rate of 6 to 9 mm root/day. When he replaced yeast extract in the culture medium by vitamin $B_1$, normal growth of excised roots was found. From these results he inferred that vitamin $B_1$ might be present in yeast extract.

Bonner and Addicott (1937) noted that vitamin $B_1$ alone was not present in yeast extract applied for maintaining normal growth of pea roots, as addition of vitamin $B_1$ alone in the culture medium in place of yeast extract was not able to support the optimal growth of excised pea roots in subsequent passages. However, a mixture of pure crystalline amino acids was capable of replacing that portion of the activity of yeast extract which was not due to vitamin $B_1$. They claimed that their medium containing vitamin $B_1$ and mixture of amino acids, was highly satisfactory for cultivation of excised pea roots as this medium of known composition supported the growth of such roots as well or better than the media containing yeast extract.

Robbins and Bartley (1937) noted that the intact molecule of thiamine could be replaced quantitatively as a growth factor of excised tomato roots by an equimolar mixture of the two fragments of the thiamine molecule (thiazole and pyrimidine). It was noted that the response of different tomato strains was varied. In some strains, thiazole replaced thiamine without adverse effect on the root, thus establishing that the addition of the pyrimidine was unnecessary.

White (1937a) observed that the material obtainable from yeast had been shown to be essential for satisfactory

growth in vitro of excised tomato roots. Optimal results were obtained with about 100 mg of yeast/l of nutrient. Treatment of yeast with various reagents showed all of the effective material to be soluble in water and 85% ethyl alcohol, stable in trichloroacetic acid, unstable in nitrous oxide and insoluble in ethyl ether. He concluded that at least 82% of yeast material was inert, only 18% in 85% alcohol extract/l of nitrient being needed for optimal result. However, separation of this material with 100% alcohol separated it into two fractions, both of which were essential for satisfactory growth. Analysis of absolute alcohol insoluble fraction showed a large amount of amino acids which was supposed to play important role as growth promoting substance for isolated tomato roots.

In another experiment, White (1937b) showed that vitamin $B_1$, a probable constituent of the yeast fraction soluble in absolute alcohol, was an important and perhaps indispensable factor in the nutrition of excised tomato roots. The growth promoting effect of vitamin $B_1$ was detectable only in the presence of accessary salts, which were also indispensable. While growth at a low level could be maintained apparently indefinitely in a nutrient medium containing only vitamin $B_1$, standard salts, accessary salts and sugar, it was notably improved by the addition of a mixture of nine amino acids.

Addicott (1939) noted that the action of vitamin $B_1$ as a growth hormone of pea root was through an effect on meristematic activity rather than cell elongation, which was the primary effect of auxin. He argued that cell elongation, differentiation and maturation proceeded normally in roots to which vitamin $B_1$ was not supplied as far as could be observed even though meristematic activity was greatly reduced.

Addicott and Devirian (1939) found that an essential growth factor for excised pea roots necessary in addition to vitamin $B_1$ and present in yeast extract was not found to be among the amino acids nor the micro-element of plant nutrition. Nicotinic acid could act as this growth factor. Salts, sugar, vitamin $B_1$ and nicotinic acid could support the growth of pea roots indefinitely.

Bonner and Devirian (1939) noted that isolated pea roots could be cultivated indefinitely in a nutrient medium containing vitamin $B_1$ and nicotinic acid in addition to mineral salts and sucrose. The growth rate in this medium was 70-85 mm/week. Vitamin $B_6$, adenine, ascorbic acid, theelin, $\beta$-alanine, pantothenic acid, vitamin E, K and $B_2$ and numerous amino acids were without effect in increasing the growth rate of isolated pea roots in the presence of vitamin $B_1$ and nicotinic acid. Isolated roots of radish were grown through fourteen passages over a period of six months at an average growth rate of 15 mm/week in a medium

containing thiamine and nicotinic acid in addition to the basic nutrients. These two vitamins were essential for the maintenance of growth. However, vitamin $B_2$, $B_6$, E and K, adenine pantothenic acid and numerous amino acids were without influence on the growth rate. On the other hand, excised flax roots responded only to $B_1$. Addition of vitamin $B_6$ or nicotinic acid was without significant effect on the growth rate. It was also shown that isolated tomato roots could be cultivated indefinitely at an average growth rate of 40 mm/week in a medium containing only vitamin $B_1$ and vitamin $B_6$. The growth rate further enhanced to 60 mm/week by supplying nicotinic acid in the medium. These studies showed that excised roots of various plants needed different growth factors.

Bonner and Greene (1939) observed that Cosmos, Pea, Brassica and Pisum, grown in sand culture under green house condition, responded to the addition of vitamin $B_1$ at 0.01 mg/l of the nutrient solution with a marked increase in the rate of dry matter accumulation. Similar response of added vitamin on dry matter accumulation of plants grown in soil was also noted. They further observed that the carob trees supplied on alternate days with vitamin $B_1$ in sand culture during first year continued to exhibit a greater growth rate than the control which did not receive the vitamin. They concluded that promotive effect of vitamin B

was lasting rather than temporary. On the other hand, tomato and Pisum did not respond to the addition of the vitamin and their leaves were noted to possess at least three and two times respectively as much vitamin than leaves of the responsive plants. They suggested that the amount of vitamin $B_1$ synthesized by leaves of the given species regulates, at least in part, the response of that species to the addition of the vitamin.

Arnon (1940), growing tomato, lettuce, cosmos, mustard and cocklebur in water culture, studied the effect of three levels, i.e. 0.0, 0.01 and 0.05 mg/l of vitamin $B_1$ on the growth of these plants. He obtained similar values for each treatment. He added that the results obtained did not support the view that, for the species investigated, intact plants grown from seeds could be benefitted by addition of vitamin $B_1$ to an otherwise favourable nutrient medium. It appeared that under the conditions of these experiments, the rate of vitamin $B_1$ synthesis was not limiting growth for any of the species investigated. He argued that seed is a storage organ for vitamin $B_1$ and other growth and food substances essential for the initial growth of the seedlings.

Hamner (1940) added vitamin $B_1$ at 0.01 mg/l in the nutrient solution once a week to a number of experimental plants. However, in experiments on cocklebur and cosmos, the vitamin was added twice and thrice a week respectively.

No visible differences were detected between the control plants and those receiving $B_1$. No significant differences were obtained in weight or in the accumulation of dry matter. The differences neither at high nor at low planes of nitrogen were detectable which could be ascribed to addition of vitamin $B_1$ to the culture. He also added that vitamin $B_1$ had no effect upon hastening flowering, upon size or number of flowers produced or upon colour or quality of the flowers of cosmos.

White (1940) noted that vitamin $B_6$ did not improve the growth of excised roots of two tomato strains in any nutrient containing adequate amount of thiamine. Also, nicotinic acid and pyridoxine did not significantly stimulate growth of the strains of tomato under similar conditions. Hence, he concluded that these two strains did not require vitamin $B_6$, nicotinic acid and pyridoxine for their root growth under the experimental conditions.

Addicott (1941) found that deficiency of vitamin $B_1$ and nicotinic acid in cultures of excised pea roots resulted in a decrease in the growth rate of roots and sometimes, cessation of the growth. It was accompanied by reduction in the length of meristem, decrease in the number of cell divisions in the meristem as well as reduction of the total length attained by the cells as they mature. He further noted that the roots deficient in nicotinic acid became thin.

This was accompanied by the reduction of the diameter of root cells and of the number of columns of cells in the roots. The meristems of roots deficient in vitamin $B_1$ became only slightly smaller in diameter in course of 4 weeks, but their growth rate declined more rapidly than did that of roots deficient in nicotinic acid. The mature portions of these roots developed irregular thicknings.

Day (1941) successfully cultured excised tomato roots in an agar nutrient medium containing 1.0% sucrose and 0.5% purified agar to which thiamine, pyridoxine nicotinamide, neopeptone, glutamic acid and glycine were added in different combinations. He found that none of the amino acids could replace the vitamin $B_6$.

Hitchcock and Zimmerman (1941) found that the application of vitamin $B_1$ did not influence the growth of potted aster plants in June or July or promoted root growth in cuttings of nine genera which had been initially treated with IBA. The cuttings of the plants used were capable of meeting their own vitamin $B_1$ requirements. However, seedlings of China aster grown in flats during February and March responded to weekly watering with a solution of thiamine by producing taller plants with a greater fresh weight than those watered with tap water only.

Minnum (1941a) grew radish and cauliflower in sand supplied with Hoagland's solution plus minor elements. He treated pots by weighing out the necessary number of mg of pure crystalline vitamin $B_1$, $B_2$ and $B_6$ and adding sufficient amount of water so that 1 ml solution contained 1 mg of vitamin. Vit Flor, a commercial compound containing 0.1% vitamin $B_1$, 0.5% nicotinic acid and traces of vitamin $B_2$, $B_6$ and pantothenic acid, was also included. It was observed that none of the treatments had any influence on these vegetables.

Minnum (1941b) treated various vegetables, namely cauliflower, muskmelon, beets, sweetcorn, summer squash, tomato, snap beans, pepper and radish with pure crystalline vitamin $B_1$, a brewers yeast containing vitamins and a commercial compound containing vitamins. He also soaked the roots of some normally transplanted plants previous to planting in a solution of IBA and then treated them with vitamins. The data showed no significane for any of the treatments.

Murneek (1941) noted that vitamin $B_1$ at concentrations of 0.025 to 1.25 mg/gallon of water had conspicuously beneficial effect on growth of several plants (common dill, ornamental pepper) in both poor and rich soil, as expressed by dry weight. He also noted that root development was influenced the most. Equally good and sometimes better

results were obtained, however, by merely putting on the surface of the soil a 1/2 to 3/4 inch layer leaf mould which supplied not only sufficient vitamin $B_1$ but undoubtedly a good number of many other organic stimulants.

Templeman and Pollard (1941) studied the effect of vitamin $B_1$ and nicotinic acid on growth and yield of spring oats and tomato in sand culture. They observed that the application of two levels each of vitamin $B_1$ and nicotinic acid and their four combinations proved ineffective and superfluous. They concluded that excellent crop could be grown by means of sand culture technique in the complete absence of organic material provided attention was paid to nutrient and water requirements.

Clark (1942) added vitamin $B_1$ at a concentration of 0.01 ppm to the nutrient solution supplied to Brassica alba grown in pure quartz sand or soil and Agroctis tenius grown in soil culture. He did not find any significant difference between the vitamin $B_1$ treated and control culture.

Robbins (1942) noted the effect of twelve analogues of pyridoxine on the growth of excised tomato roots in the presence of thiamine. Out of the twelve analogues tested, nine were inactive. Acetylation of pyridoxine and substitution of an ethyl for the methyl group in the second position on pyridoxine ring did not reduce the activity. He concluded

that pyridoxine had a high degree of specificity for the growth of excised tomato roots.

Day (1943) transferred uniform pieces of inoculum from tomato roots grown for 57 or more passages in a mineral sugar solution containing thiamine or its thiazole intermediate to petridishes, containing a modified Pfeffer's solution with 1% sucrose and 0.5% purified agar to which thiamine, pyridoxine, nicotinamide, neopeptone, glutamic acid and glycine were added in various combinations. They were incubated in moist chamber at 25°C in the dark. In basal agar with no added growth substance, the root seldom grew for more than two passages. With the addition of thiamine the root tips grew about 1.77 mm daily for an unlimited number of passages. When pyridoxine was added to the agar medium with the thiamine the daily rate was about 5.2 mm and the roots in this medium showed the characteristic hooks and curls. He further observed that supplementing this medium with nicotinamide had no appreciable effect on the rate of growth. However, further addition of glutamic acid to the agar medium containing thiamine had little or no effect.

Noggle and Wynd (1943) noted that seeds of the orchid Cattleya trianae var. mooreana x C. schroederae germinated and produced normal growth in an artificial nutrient medium in which one particular lot of maltose was applied as a source of carbohydrate but no germination of seeds or growth of

seedlings was obtained when a more purified maltose was used. It was also observed that the inability of seeds to germinate and to develop on purified maltose medium was not overcome by the addition of thiamine hydrochloride, ascorbic acid or calcium pantothenate. However, a few seeds germinated when riboflavin was added in the medium. However, the presence of pyridoxine showed good germination but subsequent germination was poor. Addition of nicotinic acid not only resulted in good seed germination but also excellent growth of seedlings.

Gisiger (1944) noted that addition of vitamin $B_1$ had no effect on the growth of sunflower, maize, wheat, flax and hericot bean in a pot experiment. He concluded that plants could elaborate tne quantities necessary for their growth. Further, there was no necessity for its presence in fertilisers.

Mariat (1944) allowed the seeds of Cattleya orchid to germinate in solution to which thiamine at the rate of 2.6 mg/l had and had not been added. It was noted that the germination was equally good in all cases but the seedlings in the tubes containing thiamine went ahead and in 3 months 42% of the embryos had produced leaves against only 3% of those not receiving the vitamin.

Almestrand (1950) tested the effect of thiamine, pyridoxine and niacin added to the solution medium on wheat roots. He noted that pyridoxine alone had a decisive effect on growth of wheat roots by promoting meristematic cell division. Optimum growth of the roots was obtained in pyridoxine concentrations ranging from 0.5 to 1.0 mg/l at 27-28$^{o}$C.

Almestrand (1951) observed the effect of pyridoxine and its derivatives (pyridoxal phosphate and pyridoxamine) on the growth and metabolism of strains of wheat, barley, oats and rye. The pyridoxine sensitive wheat variety Eroica showed marked increase in root length, whereas other varieties were not affected by the vitamin application. In order to study the metabolism, two wheat strains, namely Eroica-pyridoxine sensitive and Virtus-pyridoxine insensitive were selected. It was noted that the absorption of glucose phosphate and nitrate uptake in Eroica wheat corresponded significantly to the applied pyridoxine concentration, while Virtus did not show any response to pyridoxine treatment with regard to absorption of glucose and the ions. It was further noted that the effect of pyridoxine derivatives, i.e. pyridoxal phosphate and pyridomaxine, was similar to that of the mother compound. However, barley, oats and rye strains were unaffected.

Lee and Whaley (1953) studied the effect of individual or combined vitamin supplement of thaimin, niacin and pyridoxine to culture medium for 4 weeks on the growth of excised tomato roots. The roots were grown in culture media (a) containing no vitamins and (b) containing thiamine, niacin and pyridoxine alone and in different combinations. They noted that growth in all media was about the same during the first week. Little additional growth was obtained from roots grown in media containing pyridoxine or niacin alone. In media containing thiamine alone or combinations of any two or three of the vitamins, growth of roots was significantly greater than in the other media. In the medium containing all the three vitamins, growth of roots was significantly greater than in any other medium. However, between the third and fourth week, growth in all cultures was markedly decreased. They emphasised that the best period for investigation of growth substances effects under their experimental conditions was during the second and third weeks.

Boll (1954) observed that a clone of excised tomato roots required thiamine, pyridoxine and niacin for optimal growth but could be maintained in a medium supplemented only with thiamine and pyridoxine. He added that pyridoxine was replaceable by pyridoxal or pyridoxamine. The order of activity was pyridoxal > pyridoxine > pyridoxamine. Likewise,

niacin was replaceable by niacinamide. Nicinamide was, in general, more active. He further noted that pyridoxine could be replaced with glycine. This replacement was more pronounced in the presence than in the absence of niacin. In basal medium containing thiamine, pyridoxine and niacin, the addition of glycine at certain concentrations of pyridoxine increased level of growth to that obtained with optimal concentration of pyridoxine. Morphogenetic effects of glycine and pyridoxine were similar although glycine appeared to exert an independent effect upon the initiation of laterals. Root morphology was controlled by the balance of growth factor supplied in the medium.

Fujiwara and Ojima (1954) studied the effect of thiamine, pyridoxine and niacin on the growth of excised root tips of rice and wheat in liquid medium. The root tips of rice responded positively to thiamine or pyridoxine application. However, in case of wheat.pyridoxine gave better results. They further observed that the excised plant roots attached to their scutella grew much longer than their root tips and showed no reaction to vitamins.

Fries (1955a) observed that decotylised pea seedlings, growing in dark, needed a mixture of water soluble vitamins along with various amino acids in sucrose mineral salt medium for their optimal growth. On the other hand, excised roots were unaffected by these substances. According to him, it was because they had adequate reserves of vitamins. However,

the roots ceased to grow after one or two transfers but growth was again maintained on supplying the niacin and thiamine. But, on comparison of the medium lacking vitamins, the growth of pea seedlings was accelerated by addition of thiamine and pyridoxine. However, niacin showed either poor or no effect.

Fries (1955b) observed that in a sucrose mineral salt nutrition medium supplemented with arginine, glycine and adenine, the two vitamins, i.e. thiamine and pyridoxine proved the chief limiting factor for decotylised pea seedlings grown in darkness. To maintain normal growth of the main root, 10 µg/l thiamin and 100 µg/l pyridoxine were needed. It was noted that even in the light, the addition of thiamine controlled the rate of growth to a certain degree during the experiment.

Barbieri (1959) studied the effect of vitamin $B_1$ and $B_6$ on pea, broad bean, beet and wheat plants in pot trials. He added vitamin $B_1$ and $B_6$ with various media. He observed that the vitamin application increased plant height, leaf number and fresh and dry weight of the plants. He added that the response was considerable in beets but less in pea and broad beans. For example, in unsterilised garden soil, these vitamins at 0.01 mg/l increased the leaf number in beet seedlings to five compared with four in control.

Skol' Nik and Davydova (1962) observed that tomato plants grown in a nutrient solution without zinc showed severe deficiency symptoms but when vitamin $B_1$ and $B_6$ each at 100 mg/l were added the symptoms disappeared to a large extent and the plant resembled those grown in a solution with zinc.

Dimitrova-Russeva and Lilova (1969) grew Mentha piperata plants both in nutrient solution and in soil. Application of thiamine, pyridoxine and nicotinic acid stimulated the uptake of nitrogen and phosphorus. Single application of nicotinic acid and double application of the other two vitamins were most effective in stimulating the removal of phosphorus by the plants. Application of nicotinic acid also increased the yield of essential oil as did application of thiamine whereas, pyridoxine reduced it.

Galzy (1969) pointed out that the cuttings of Vitis rupestris with a single leaf grown in culture medium needed sucrose for both root and shoot growth. At 20°C, the presence of vitamins of B group improved root growth, whereas at 35°C, they proved essential for both root and shoot growth.

Maroti (1969) reported that the uptake of thiamine from a solution supplemented with thiamine in various concentrations by the excised tobacco tissue was not connected with

the increase in weight of the tissue. He also noted a correlation between the uptake and thiamine content of the soil. The thiamine concentration was not a limiting factor in tissue growth because it was not totally incorporated by the cells. Kinetin without IAA did not increase the weight of the tobacco tissue inspite of its alleged stimulating effect on growth. Above a certain concentration, however, it inhibited thiamine uptake. He concluded that thiamine stimulated tissue growth only in conjunction with kinetin and IAA.

Zavenyagina and Bukin (1969) investigated the effect of application of an antagonist of pyridoxine on the germination, viability of seeds, development of the root system and above ground portion of pea and wheat seedlings in water culture. They observed that the pyridoxine antagonist introduced into Knop's nutrient medium at $10^{-3}$ to $10^{-5}$M lowered the germination rate and suppressed growth of roots and above ground portion of the seedlings of both species. The appearence of symptoms of $B_6$-avitaminosis (growth lag and chlorosis) was partially or entirely prevented by the addition of pyridoxine to the culture medium. They also observed that vitamins of $B_6$ group stimulated the growth of normal wheat and pea seedling and increased chlorophyll content in the leaves.

Kozhin and Kravtsov (1973) added pyridoxine to Simurnous medium in concentration of 0.001, 0.01, 0.1, 1.0 and 10 mg/l to study its effect on isolated pear and apple embryos at different stages of ripeness. They noted that pyridoxine enhanced germination, more accumulation of chlorophyll and differentiation of embryos into seedlings. The embryos from unripe seeds were more affected by pyridoxine than those from ripe seeds.

Peichl and Trojan (1975) dipped the cuttings of the five clones from three cultivars of Medicago sativa in all combinations of 0, 25, 50, 75, 100 or 250 ppm indole-3-acetic acid (IAA) and 0, 25, 50, 75 or 100 ppm thiamine for 24 h. They noted that rooting was highest with 75 ppm IAA and thiamine. However, there were no differences in the effect of various concentrations of thiamine used.

Ohira et al. (1976) found that the omission of thiamine from the medium led to a rapid and complete cessation of growth in sub-cultures of soybean, tobacco and rice cells. The critical levels of thiamine contents in these cells were considered to be in the vicinity of 0.6, 0.5, and 0.2 µg/g dry weight respectively. Soybean cells could grow satisfactorily when supplied with an equimolar mixture of two thiamine precursors, pyrimidine and thiazole. On the other hand, Ruta and peanut cells could be successfully subcultured for ten passages in the absence of externally

supplied thiamine. When grown with thiamine, Ruta cells had an average thiamine content of 0.5-0.6 µg in darkness and 3.5 µg/g dry weight in the light.

Pandev (1979) noted the effect of plant treatment with vitamin $B_1$ on nitrogen absorption by wheat in sand culture. Plants were supplied with complete Hoagland and Arnon solution. Application of vitamin $B_1$ increased the uptake of nitrogen at the period of reduced nitrogen uptake (flowering stage).

Marino (1983) observed that dormant buds excised from woody shoots of the Japanese plum cultivar Santa Rosa in late winter proliferated well when grown on Murashige and Skoog's medium containing 1.5 mg/l thiamine hydrochloride, 2 mg/l glycine, 0.5 mg/l pyridoxine hydrochloride, 0.5 mg/l nicotinic acid, 0.1 mg/l $GA_3$, 0.1 mg/l IAA or 0.05 mg/l NAA and 1-2 mg/l BA. When the shoots were transferred to a medium containing 5 or 10 mg/l 2iP in place of BA, the proliferation rate decreased but shoot elongation increased. Proliferation was enhanced by five milli moles/l P but there was some leaf yellowing. The best rooting (80-100%) was obtained in media containing 0.2-0.5 mg/l IBA or 0.2 mg/l NAA and half strength mineral salts. He added that rooted plants were successfully transferred to soil.

Rao and Reddy (1985) allowed Vigna radiata var. PS-16 seeds to grow in petridishes containing nutrient solution

to which various B group vitamins (thiamine, riboflavin, pantothenic acid, pyridoxine, nicotinic acid and biotin) were added individually at a concentration of 20 mg/l each. They observed a favourable effect of B-vitamins on the absorption of calcium, sodium, potassium and phosphorus by seedlings. Pyridoxine, pantothenic acid and nicotinic acid showed profound influence on the absorption of potassium and phosphorus, while thiamine, riboflavin and biotin influenced their absorption slightly.

2.4.3 Foliar treatment

Leaves are the seat for the synthesis of many vitamins, including B-vitamins. If the level of vitamin/s produced in the leaves is insufficient for the optimum performance of the crop, foliar application of the vitamin/s may prove effective in enhancing the yield of economically valuable ingredients of the crop considerably. Vitamins can be sprayed on foliage easily with foliar application of pesticides and nutrients. Keeping this in mind, scientists have done a number of experiments on foliar application of vitamins to study their effect on growth and differentiation of many plants. The available literature is reviewed below:

Iijima (1955) sprayed thiamine solution on the leaves of five varieties of sweet potatoes. He observed that its application increased the number and length of the root;

fresh weight and percentage of large storage roots. Spraying of the vitamins in the first half of the growing season proved more effective than in the latter half. The most effective thiamine concentration for spraying was 1 ppm when 2 ml of the solution was sprayed on one plant at intervals of 10 days.

Iijima (1956a) studied the effect of foliar application of thiamine on respiration of potato, sweet potato, and kidneybeans. He observed that the respiration of leaves and roots was increased by moderate concentrations ( 1 ppm and 100 ppm). In treated leaves, the respiratory quotient was approximately unity. He therefore, concluded that thiamine promoted foliar decomposition of carbohydrates.

Further, Iijima (1956b) noted the effect of foliar application of thiamine on chemical composition of Masterpiece beans, potatoes and sweet potatoes. Sprays of thiamine slightly enhanced the sugar, starch and thiamine contents of bean seeds. They (sprays) increased the carbohydrate content and C/N ratio of stem, leaves and roots. He concluded that the treatment promoted root development and flower bud differentiation and shortened the growing period.

Boukin (1958) found that growth of beans, tobacco, tomato and mulberry was stimulated spraying with 0.001% nicotinic acid or thiamine. The same treatment to young

carrots increased leaf growth and enhanced root yield by more than 50%.

Kudrev and Pavlov (1965) flooded Bulgaria No 301 cv. of wheat at (a) tillering (b) shooting and (c) heading stage of growth. They found that the flooding affected growth and development, particularly at the later stages and reduced yield by decreasing the number and weight of grains. However, foliar application of aqueous $B_6$ solution to the flooded plants increased grain production by restoring the disturbed nitrogen metabolism, particularly when very little damage had been done.

Artamonov (1966) studied the effect of gibberellic acid and vitamin $B_2$ applied to the leaves of sugarbeet. Gibberellin at 20 mg/l inhibited the synthesis of chlorophyll and increased its decomposition in leaves of sugarbeet. However, in association with vitamin $B_2$, it enhanced the chlorophyll content and did not show these ill effects.

Kudrev and Pandev (1967), while studying the nitrogen uptake and the content of total and protein nitrogen in leaves of wheat cv. Mercury in nutrient solution at different stages, noted that sprays of vitamin $B_1$ and $B_6$ stimulated nitrogen uptake temporarily but did not change the general pattern of uptake.

Popova et al. (1971) observed that sprays of solutions of thiamine, riboflavin, pyridoxine and nicotinic acid in various combinations on the pistils of Capsicum annuum cv. Kalinkov-805 just after pollination increased fruit set and the number of seeds/fruit, shortened the growing period; and increased plant height in the $F_1$ generation.

Kulieva et al. (1976) sprayed melon cv. Sary Gulyabi and watermelon cv. Zimnu-344 with thiamine, cyanocobalamine, nicotinic acid, pyridoxine or ascorbic acid at concentrations ranging from 0.01 to 0.0001%. Generally, the best results were obtained by spraying the plants with cyanocobalamine (0.001%) or ascorbic acid 0.01%.

Arsene'va (1977) sprayed vitamin $B_1$, vitamin C and nicotinic acid mixture at 100 mg/l and vitamin $B_6$ at 100 mg/lon Syringa vulgaris, Hydrangea pannicuta and Spiraea callosa at reproductive organ differentiation and at flower bud development. The effectiveness of the treatments was determined by the start and end of flowering, flower size and colour and shoot growth. It was found that the mixture of the vitamins proved optimum for Spiraea callosa and vitamin $B_6$ for Syringa vulgaris.

Reda et al. (1977) noted that foliar spray of thiamine at 50 mg/l promoted root, stem, leaf, umbel and fruit growth of Ammi visnaga.

El-Kholy and Saleh (1980) studied the effect of spray of thiamine on the yield of essential oil and chamazulene formation in Matricaria chamomilla. Uniform M. chamomilla seedlings were transplanted in pots and sprayed (10 ml/plant) with an aqueous solution of thiamine at concentration of 25, 50, 100 and 150 ppm at 30 and 45 days after transplantation. Application of the vitamin at 150 ppm significantly increased flower head production/plant over that of the control by 47.22% for the first growing season. On the other hand, vitamin $B_1$ did not influence the percentage of essential oil and chamazulene. The spray of vitamin $B_1$ at a concentration of 150 ppm resulted in an average increase of 39.2% in essential oil content and 42.34% in chamazulene content over the untreated plant.

Samiullah et al. (1985) conducted a field experiment Vigna radiata var. K-851. They applied aqueous solution of pyridoxine at 0.0, 0.1, 0.2, 0.3, 0.4 and 0.5% as foliar spray on the leaves of the crop either at the pre-flowering (35d) or at the post-flowering (45d) stage. They measured nitrate reductase activity (NRA) 10 days after each spray. The spray of 0.1% pyridoxine solution at the flowering stage and 0.2% at the post-flowering stage proved to be optimal and NRA was found to be increased by 49.2 and 29.6% respectively over the water-sprayed control. NRA estimated at 55 d was significantly correlated with seed yield. Thus,

they concluded that estimation of NRA is a rapid and reliable technique for predicting crop productivity.

Ansari et al. (1985) applied graded pyridoxine solution 0.0, 0.025, 0.05, 0.1, or 0.2% to Vigna radiata (var. K-851) leaves either at pre-flowering (35d) or at post-flowering (45d) stage in the field. The treatment significantly enhanced leaf nitrate reductase activity (NRA) at 45 and 55d after sowing, with 0.1% proving optimum. The 0.1% spray increased seed yield by 27% and 23% and protein content by 3% and 2.5% in plants sprayed at 35 and 45d respectively. They concluded that leaf NRA level could be used to predict seed yield and quality as NRA at early growth stage was significantly correlated with seed yield and protein content.

Ansari et al. (1987) noted the effect of foliar spray of aqueous solution of pyridoxine (0.0, 0.1, 0.2, 0.3, 0.4 and 0.5%) at 90 or 110d on growth and yield of lentil var. T-36 in a field trial. They found that foliar spray of 0.2% pyridoxine solution at 90d optimally enhanced all growth parameters (plant length, root length, root nodule number, leaf number, fresh weight and dry weight at 120d and net assimilation rate for tne period of 90-120d). However, the seed yield and seed protein content showed variable response to pyridoxine spray. For example, pods/plant, seeds/pod and seed yield were optimum in 0.2% pyridoxine treatment applied 90d, being 35.6, 3.9 and 14.0% respectively higher than in

the control, while pod length was maximum in plants sprayed with 0.2% pyridoxine solution at 110d and seed protein content, in 0.1% pyridoxine sprayed at 90d being 3.5 and 1.6% respectively more than in water sprayed control.

Ansari (1988) applied foliar spray of 0.0, 0.025, 0.05, 0.1, and 0.2% aqueous pyridoxine solution on field grown Vigna radiata var. K-851 either at flower initiation (35d) or at pod initiation (45d) stage. Among foliar treatments, 0.1% pyridoxine solution applied at flower initiation stage invariably proved optimum for plant height, root length, leaf number, fresh weight and dry weight at 45 and 55d and net assimilation rate for the periods 35-45 and 45-55d. However, root nodule number was found maximum as a result of 0.1% treatment at 45d.

2.4.4 Combined pre-sowing seed and foliar treatment

As noted in the studies in preceding pages, pre-sowing seed treatment or foliar treatment with aqueous solution of vitamins resulted in increased growth and development of plants. Therefore, it is desirable to study their effect in combination. However, the literature in this regard is very meagre. Only some research work has been done at Aligarh and is reviewed below:

Ansari (1986) observed the effect of soaking the seeds Vigna radiata var. K-851 for 4h in 0.0 ($S_w$) and 0.3% ($S_3$)

and foliar spray of 0.0 ($F_W$), 0.1 ($F_1$), 0.2 ($F_2$) and 0.3% ($F_3$) aqueous pyridoxine solution at 35 and 45d after sowing in different combinations $S_W+F_W(35)$, $S_3+F_W(35)$, $S_3+F_1(35)$, $S_3+F_2(35)$, $S_3+F_3(35)$, $S_W+F_W(45)$, $S_3+F_W(45)$, $S_3+F_1(45)$, $S_3+F_2(45)$ and $S_3+F_3(45)$ on plant length and root nodule number at 45d and on fresh weight, dry weight, net assimilation rate, nitrate reductase activity and leaf NPK content at 45 and 55d and net assimilation rate for 35-45d and 45-55d in a simple randomised field experiment. Among different treatments, $S_3+F_W(35)$ and $S_3+F_W(45)$, being at par in their effect, proved optimum for most parameters.

In 1986, Ansari conducted another field experiment on lentil var. T-36 to study the combined effect of soaking the seeds for 12h in 0.0 ($S_W$), 0.2($S_2$), 0.3($S_3$) and 0.4%($S_4$) and foliar spray of 0.0($F_W$), 0.1($F_1$) 0.2($F_2$) and 0.3%($F_3$) aqueous pyridoxine solution at 90d on plant length, root length, root nodule number, leaf number, fresh weight, dry weight nitrate reductase activity and leaf NPK content at 120d; NAR for 90-120d; pods/plant, pod length, seeds/pod, 1,000 seed weight, seed yield and seed protein content at harvest. The soaking and foliar spray combinations included $S_W+F_W$, $S_W+F_1$, $S_W+F_2$, $S_W+F_3$, $S_2+F_W$, $S_2+F_1$, $S_2+F_2$, $S_2+F_3$, $S_3+F_W$, $S_3+F_1$, $S_3+F_2$, $S_3+F_3$, $S_4+F_W$, $S_4+F_1$, $S_4+F_2$ and $S_4+F_3$. Of the various combinations, $S_3+F_W$ proved optimum for most parameters, including seed yield.

Khan (1989) studied the effect of soaking the seed of Vigna radiata var. K-851 for 4h in 0.0 ($S_W$) and 0.3% ($S_3$) and foliar spray of 0.0 ($F_W$), 0.1($F_1$), 0.2($F_2$) and 0.3%($F_3$) aqueous pyridoxine solution at 35 or 45d after sowing in different combinations $S_W+F_W(35)$, $S_3+F_W(35)$, $S_3+F_1(35)$, $S_3+F_2(35)$, $S_3+F_3(35)$, $S_W+F_W(45)$, $S_3+F_W(45)$, $S_3+F_1(45)$, $S_3+F_W(45)$, $S_3+F_1(45)$, $S_3+F_2(45)$ and $S_3+F_3(45)$ on pods/plant, pod length, seeds/pod, 1,000 seed weight, seed yield and protein yield in a simple randomised field experiment. Of the treatments, $S_3+F_W(35)$ and $S_3+F_W(45)$ proved optimum for all characters.

## 2.5 Concluding remarks

The above review of literature broadly establishes that B-vitamins have stimulating effect on growth and development of plants. Of the various methods of vitamin application, pre-sowing seed treatment seems to be promising due to many factors, including the small amount of the vitamins required and low operation cost involved. The literature also imparts that comparatively less work has been done on mustard. It is therefore, highly desirable to extend the work by soaking the seeds of new selected varieties of mustard in aqueous solutions of vitamins of varying concentrations.

# CHAPTER - 3

# MATERIAL AND METHODS

# MATERIAL AND METHODS

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# CHAPTER - 3

# MATERIAL AND METHODS

To attain the objectives pointed out at the end of the last chapter, two sand culture experiments will be conducted on mustard during 'rabi' (winter) seasons of two consecutive years at the Botanical Garden of the Aligarh Muslim University. The details of material and methods are given below:

## 3.1 Seeds

Authentic seeds of selected mustard varieties will be obtained from the I.A.R.I., Regional Research Station, Kanpur. Seeds of uniform size will be selected and their viability tested. The seeds will be surface sterilised with ethyl alcohol before sowing.

## 3.2 Sand

Purification of sand will be done according to the method given by Hewitt (1966). The coarse sand will be washed first and then treated with 18% hydrochloric acid (HCl) for 24h. After treating it with HCl, the sand will be washed thoroughly with tap-water. Final washing will be done with deionised water.

## 3.3 Pots

Earthen pots of requisite size will be used. The inner wall of the pot will be covered with polythene sleeves, and the lower end of the polythene will be passed through the hole at the bottom of the pot to ensure drainage and aeration. Each pot then will be filled with the purified sand. The pots will be kept in a net house.

## 3.4 Experiment 1

The aim of this experiment will be to select the most efficacious B-vitamin as well as most responding variety on the basis of yield and quality characters.

The experiment will be carried out on three varieties of mustard (low, medium and high yielding). One gram healthy seeds of each variety will be soaked in 10 ml of water (control) and 0.01% aqueous solutions of eight B-vitamins (thiamine, riboflavin, pantothenic acid, nicotinic acid, pyridoxine, biotin, folic acid and cyanocobalamine) for 4h.

Five treated seeds of each variety will be sown in each pot at the depth of 2 cm. After germination, only two plants will be allowed to grow till maturity. Each treatment will be replicated five times. The scheme of the experiment is given below:

Scheme

| Vitamins | Varieties according to yield | | |
|---|---|---|---|
| | Low | Medium | High |
| Thiamine | " | " | " |
| Riboflavin | " | " | " |
| Pantothenic acid | " | " | " |
| Nicotinic acid | " | " | " |
| Pyridoxine | " | " | " |
| Biotin | " | " | " |
| Folic acid | " | " | " |
| Cyanocobalamine | " | " | " |

Each pot will be provided with complete Long Ashton nutrient solution (Hewitt, 1966) or water as and when required. At harvest, yield and quality parameters will be studied. The analysis of the data will be done according to factorial randomised design.

3.5 Experiment 2

The aim of the experiment will be to determine the optimum concentration of the most efficacious vitamin, taking the most responding variety on the basis of the data of Experiment 1.

One gram seeds of the selected variety will be soaked separately in water (control), 0.025, 0.05, 0.1, 0.2 and 0.4% aqueous solutions of the vitamin for 4h. Five soaked

seeds will be sown in each pot. After germination, only two plants will be maintained in each pot. Other cultural practices will be the same as adopted in Experiment 1. Growth, NPK content and carbonic anhydrase activity will be studied at 50, 70 and 90 days after sowing. Net assimilation rate will be determined for the period 50-70 and 70-90 days after sowing. Yield and quality parameters will be studied at harvest. There will be five replicates for each characteristics. The analysis of the data will be done according to a simple randomised design.

### 3.6 Nutrient solution

Stock solutions (Table 1) of essential macro- and micro-nutrients will be prepared according to Hewitt (1966). The stock solutions will then be diluted as per Table 2 for watering the plants.

Table 1. Percentage of the various macro- and micro-nutrients furnishing salts in stock solutions

| Salts | Quantity (g) in 100 ml stock solution |
|---|---|
| Macro-nutrients | |
| $Ca(NO_3)_2$ (anhydrous) | 32.0 |
| $KNO_3$ | 20.2 |
| $MgSO_4 . 7H_2O$ | 18.4 |
| $NaH_2PO_4 . 2H_2O$ | 20.8 |
| $FeC_6H_5O_7 . 3H_2O$ | 5.98 |

| Micro-nutrients | |
|---|---|
| $MnSO_4 \cdot 4H_2O$ | 2.230 |
| $CuSO_4 \cdot 5H_2O$ | 0.250 |
| $ZnSO_4 \cdot 7H_2O$ | 0.290 |
| $H_3BO_3$ | 1.860 |
| $(NH_4)_6 Mo_7O_{24} \cdot 4H_2O$ | 0.088 |

Table 2. Preparation of the nutrient solution from stock solutions for watering

| Stock solutions | Volume (ml) |
|---|---|
| (a) Macro-nutrients | |
| $Ca(NO_3)_2$ (anhydrous) | 2.0 |
| $KNO_3$ | 2.0 |
| $Mg.SO_4 \cdot 7H_2O$ | 2.0 |
| $NaH_2PO_4 \cdot 2H_2O$ | 1.0 |
| $FeC_6H_5O_7 \cdot 3H_2O$ | 0.5 |
| (b) Micro-nutrients | |
| Mixed stock solutions of the salts for micro-nutrients | 0.1 |
| Deionised water | 992.4 |
| Total volume (ml) of the nutrient solution for watering | 1000.0 |

## 3.7 Sampling technique

The two plants in each pot will form the sample.

### 3.7.1 Growth characteristics

The following growth parameters will be studied in the five replicates of the sample and converted to get data for single plant:

i) Height of plant (cm)

ii) Root length (cm)

iii) Leaf number

iv) Area/leaf ($cm^2$)

v) Total fresh weight (g)

vi) Fresh weight of shoot (g)

vii) Fresh weight of root (g)

viii) Total dry weight (g)

ix) Dry weight of shoot (g)

x) Dry weight of root (g)

xi) Net assimilation rate ($g/cm^2/d$)

#### 3.7.1.1 Determination of net assimilation rate

Net assimilation rate is defined as the increase in weight per unit leaf area per unit time. It is represented by $E_L$. It is expressed as grammes per square decimeter of leaf area per week. It is estimated by the formula:

$$\frac{W_2 - W_1}{t_2 - t_1} \times 2.303 \times \frac{Log_{10}(a_2 - a_1)}{a_2 - a_1}$$

Where $W_1$ and $W_2$ are dry weights of plant; $a_1$ and $a_2$ are leaf areas of plant; and $t_1$ and $t_2$ are days of sampling at first and second growth stage respectively.

### 3.7.2 Estimation of nitrogen, phosphorus and potassium in leaf

The plant samples will be allowed to dry in an oven at 80°C for 24h. After taking their dry weight, the blades of the leaves will be finely powdered. The leaf powder will be passed through a 70 mesh screen and stored in polythene vials, if required.

#### 3.7.2.1 Digestion

100 mg of the dried leaf powder of each sample will be transferred to a 50 ml Kjeldahl flask to which 2 ml sulphuric acid will be added. The contents of the flask will be heated on atemperature controlled assembly for about 2 h to allow complete reduction of nitrates present in the plant material by the organic matter itself. The contents of the flask will turn black. After cooling the flask for about 15 minutes, 0.5 ml 30% hydrogen peroxide will be added drop wise and the solution will be heated again till the colour of solution changes from black to light yellow. After heating for about 30 minutes, the flask will be allowed to cool for 10 minutes and an additional amount of 3-4 drops of 30% hydrogen peroxide will be added, following heating for another 15 minutes. The addition of 30% hydrogen peroxide, following heating will be repeated if the contents of the flask do not become colourless. The peroxide digested material will be transferred from the Kjeldahl

flask to a 100 ml volumetric flask with three washings with 5 ml double distilled water (DDW) each and the volume of the volumetric flask will be made upto the mark with DDW. A proper aliquot will be used for the estimation of nitrogen, phosphorus and potassium as described below.

3.7.2.2 Estimation of nitrogen

Estimation of nitrogen will be carried out by the method of Lindner (1944). A 10 ml aliquot of the above digest will be taken in a 50 ml volumetric flask to which 2 ml of 2.5 N sodium hydroxide will be added to neutralise the excess of acid. 1 ml of 10% sodium silicate will also be added to the flask to prevent turbidity and the volume, made upto the mark with the help of DDW. 5 ml of this solution will be taken in a test tube to which 0.5 ml Nessler's reagent will be added drop by drop. The contents of the tube will be allowed to stand for 5 minutes for maximum colour development. The solution will be transferred to a colorimetric tube and the optical density (O.D), read at 525 nm with the help of a spectrophotometer. A blank will be run with each determination. A standard curve will be obtained taking known dilutions of ammonium sulphate solution versus O.D. The reading (O.D.) of each sample will be compared with this calibration curve.

3.7.2.3 Estimation of phosphorus

The phosphorus content will be estimated by the method of Fiske and Subba Row (1925). A 5 ml aliquot will be taken in a test tube to which 1 ml molybdic acid reagent (2.5% ammonium molybdate in 10N $H_2SO_4$) will be added, followed by the addition of 0.4 ml 1-amino-2-naphthol-4-sulphonic acid, which will turn the contents blue. The contents of the tube will be allowed to stand for 5 minutes for maximum colour development. Tney will be transferred to a colorimetric tube and per cent transmittance will be read at 620 nm. A blank will be run with each reading. A standard curve will be prepared, using known concentrations of monobasic potassium phosphate solution.

3.7.2.4 Estimation of potassium

Potassium will be estimated flame photometrically taking a 1 ml aliquot. A blank (distilled water) will be run side by side. The reading will be compared with a calibration curve plotted for different dilutions (0-10 ppm) of potassium, using potassium chloride versus readings on the scale of the galvanometer.

3.7.3 Estimation of carbonic anhydrase activity

Carbonic anhydrase activity in the leaves will be determined according to the method of Dwivedi et al. (1974).

Carbonic anhydrase enzyme catalyses the reversible hydration of $CO_2$ to give bicarbonate ion -

$$H_2O + CO_2 \underset{\text{carbonic anhydrase}}{\rightleftharpoons} H^+ + H\,CO_3^-$$

The green leaves of the sampled plants will be cut into pieces (1 cm length) at a temperature below 25°C. After mixing these pieces, 400 mg leaves will be taken and further cut into small pieces (2-3 mm) in 10 ml of 0.2 M cystein solution in a petridish at 0-4°C. After cutting, they will be blotted to remove the solution adhering at the surface and transferred immediately to a test tube having 4 ml of 0.2 M phosphate buffer (pH 6.8), 4 ml 0.2 M sodium bicarbonate in 0.02 M NaOH solution and 0.2 ml 0.002% bromothymol blue indicator. After shaking, the tube will be kept at 0-4°C for 20 seconds and incubated at 0.4°C for 10 minutes.

$CO_2$ liberated during catalytic action of enzyme on $NaHCO_3$ will be estimated by titrating the reaction mixture against 0.05N HCl, using methyl red indicator. Control reaction mixture will also be titrated and difference between sample reading and control reading will be taken.

$$\text{Calculation: } \frac{V \times N \times 22}{200}$$

Where, V is the volume difference between control and incubated enzyme (HCl used), N/200 is the normality of HCl and 22 is the equivalent weight of $CO_2$.

### 3.7.4 Yield characteristics

The following yield parameters will be studied:

(i) Number of pods/plant

(ii) Number of seeds/pod

(iii) Hecto-litre weight of seeds

(iv) Seed yield

(v) Oil percentage

(vi) Oil yield

### 3.7.5 Determination of oil content

#### 3.7.5.1 Grinding

In order to extract the oil, grinding of seeds will be done until the formation of a fine meal.

20 g ground seeds (fine meal) will be transferred to a Soxhlet apparatus and sufficient quantity of pure petroleum ether will be added. The apparatus will be kept in a hot water-bath, running at 60°C for about 6h for complete extraction of the oil. The petroleum ether will be evaporated from the extract. The percentage of the extracted oil will be calculated by the following formula:

$$\frac{m \times 100}{m_o}$$

Where, m is the sum of the mass of oil and $m_o$ is mass of seed powder in g.

3.7.6 Quality characteristics

Three quality characters will be studied in the seeds.

(i) Acid value

(ii) Iodine value

(iii) Saponification value

3.7.6.1 Determination of acid value

The acid value of an oil is the number of mg of potassium hydroxide (KOH) required to neutralise free acid in 1 g of the oil. It will be determined by the following method (Anonymous, 1970).

2 g oil will be dissolved in 50 ml solvent mixture of 95% ethanol and diethyl ether (1:1) in a 250 ml conical flask. Titration will be carried out with 0.1N KOH solution, using phenolphthalein as an indicator and the number of ml (a) of 0.1 N KOH required will be noted. The acid value will be calculated by the following formula:

$$\text{Acid value} = \frac{a \times 0.00561 \times 1000}{w}$$

Where, a is the number of ml of 0.1N KOH required and w, the weight of oil in g.

3.7.6.2 Determination of iodine value

The iodine value of an oil is the number of g of iodine absorbed by 100 g of oil and expressed as the weight

of iodine. It will be determined by iodine monochloride method given below.

2 g oil will be taken into a dry ground neck flask and 10 ml carbon tetrachloride ($CCl_4$) and 20 ml iodine monochloride solution (ICl) will be added.

The flask will be stoppered and allowed to stand in a dark place for about 30 minutes. After that 15 ml of potassium iodide (KI) solution and 100 ml of double distilled water will be poured with proper shaking. Titration will be carried out with 0.1N sodium thiosulphate ($Na_2S_2O_3$) solution, using starch solution as an indicator. Number of ml 'a' of sodium thiosulphate solution will be noted. Side by side, the same operation will be carried out without the oil and the number of ml 'b' of 0.1N sodium thiosulphate solution will be noted. Iodine value will be calculated by the following formula (Anonymous, 1970).

$$\text{Iodine value} = \frac{(b-a) \times 0.01269 \times 100}{w}$$

Where, 'a' and 'b' are the numbers of ml of 0.1N sodium thiosulphate solution used in a sample and blank titration respectively and 'w' is the weight of oil in g.

3.7.6.3 Determination of saponification value

The saponification value of an oil is the number of mg of KOH required to neutralise the fatty acids resulting from the complete hydrolysis of 1 g of the oil.

2 g oil will be taken in a 250 ml conical flask and 25 ml 0.5N KOH solution will be added. The flask will be attached with reflux condenser and boiled on water-bath for about 1h with frequent rotation of the contents of the flask. After cooling, 1 ml phenolphthalein solution will be added. The excess of alkali will be titrated with 0.5N HCl. The number of ml (a) of 0.5N HCl will be noted. The same operation will be repeated without oil and the number of ml (b) required will be noted (Anonymous, 1970).

Saponification value will be calculated by the following formula:

$$\text{Saponification value} = \frac{(b-a) \times 0.02805 \times 1000}{w}$$

Where 'a' and 'b' are the numbers of ml of 0.5N HCl used in sample and blank titration, respectively and 'w' is the weight of oil.

## 3.8 Statistical analysis and discussion

The experimental data will be statistically analysed by adopting analysis of variance technique according to the design of the experiment (Panse and Sukhatme, 1985). In applying the 'F' test, the error due to replicates will also be determined. When 'F' value will be found significant at the 5 per cent level of probability, critical difference (C.D.) will be calculated and correlation between various parameters will be worked out. The significant data will be discussed in the light of the findings of other researchers.

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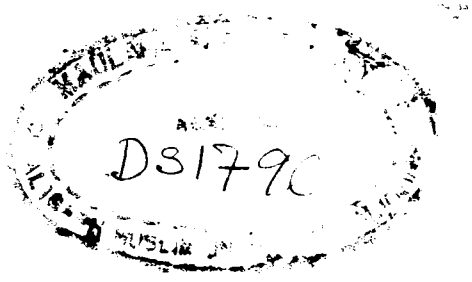

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# APPENDIX

# APPENDIX

Procedures for the preparation of reagents to be used for bio-chemical analysis are as follows:

1. Reagents for determination of N, P and K content of leaves

1.1 Nessler's reagent

(a) 3.5 g potassium iodide will be dissolved in 100 ml distilled water to which 4% mercuric chloride solution will be mixed with continued stirring till a slight red precipitate remained, about 325 ml of the solution will be required.

(b) 120 g of sodium hydroxide will be dissolved in water and the final volume will be made upto 250 ml.

Solution (a) and (b) will be mixed together and the final volume will be made upto 1,000 ml. This solution will be stored in an amber coloured bottle in a refrigerator.

1.2 Molybdic acid reagent (2.5%)

6.25 g of ammonium molybdate will be dissolved in 75 ml $10NH_2SO_4$. To this solution, 175 ml distilled water will be added in order to get 250 ml of the above reagent.

1.2.1 Sulphuric acid (10 N)

27.2 ml sulphuric acid will be added carefully to distilled water and final volume will be made upto 100 ml.

1.3 Aminonaphthol sulphonic acid

0.5 g 1-amino-2 naphthol-4-sulphonic acid will be dissolved in 195 ml of 15% sodium bisulphite solution to which 5 ml of 20% sodium sulphite solution will be added. The above solution will be stored in a dark coloured bottle in a refrigerator.

2. Reagents for determination of acid iodine and saponification value of oil

2.1 Hydrochloric acid (0.5 N HCl)

Hydrochloric acid (21.49 ml) will be mixed with 478.51 ml of double distilled water (DDW) to get 500 ml of 0.5 N HCl.

$$\text{Normality of an acid} = \frac{\text{Percentage of acid X specific gravity X 10}}{\text{Equivalent weight of acid}}$$

2.2 Iodine monochloride solution (ICl)

Iodine (13 g) will be dissolved in a mixture of 300 ml of carbon tetrachloride and 700 ml of glacial acetic acid and the resulting solution will be divided in to solutions A and B. To solution A (20 ml), 15 ml of potassium iodide and 100 ml of DDW will be added and titrated against

0.1 N sodium thiosulphate solution ($Na_2S_2O_3$), using starch as an indicator. Chlorine gas will be passed through solution until the amount of 0.1 N $Na_2S_2O_3$ required for the titration will not be more than the double of that needed in solution A.

2.3 Phenolphthalein solution

Phenolphthalein (1 g) will be dissolved in 95% ethanol and the volume will be made upto 100 ml.

2.4 Potassium hydroxide solution (0.1 N KOH)

Potassium hydroxide (5.6 g) will be dissolved in 95% ethanol and the volume will be made upto one litre.

2.5 Potassium hydroxide solution (0.5 N KOH)

Potassium hydroxide (28 g) will be dissolved in 95% ethanol and the volume will be made upto one litre.

2.6 Potassium iodide solution (KI)

Potassium iodide (150g) will be dissolved in DDW and the volume will be made upto one litre.

2.7 Sodium thiosulphate solution (0.1 N $Na_2S_2O_3$)

Sodium thiosulphate (24.8 g) will be dissolved in DDW and the volume will be made upto one litre.

2.8 Solvent mixture

Ethanol (95%) will be mixed with diethyl ether in 1:1 ratio. This mixture of solvent will be neutralised, just before use, by means of 0.1 N KOH solution in the presence of phenolphthalein solution as an indicator.

2.9 Starch solution

Soluble starch (1 g) will be dissolved in 100 ml of boiling DDW.

3. Reagents for estimation of carbonic anhydrase

3.1 0.2 M $NaHCO_3$ in 0.02 M NaOH

16.8 g sodium bicarbonate will be dissolved in sodium hydroxide solution (0.8 g NaOH/l) and its volume will be made 1 litre by the same solution.

3.2 0.002% bromothymol blue indicator in ethanol

This will be prepared by dissolving 0.002 g bromothymol blue in approximately 100 ml ethanol.

3.3 0.2 M cystein

48 g of cystein will be dissolved in distilled water and its volume will be made 1 litre.

3.4 0.2 M phosphate buffer-6.8 pH

It will be prepared by dissolving 27.8 g/l monobasic sodium phosphate and 53.65 g/l of diabasic sodium

phosphate ($Na_2HPO_4.7H_2O$). To achieve 6.8 pH, 5 ml of monobasic sodium phosphate solution will be mixed with 49 ml of diabasic sodium phosphate solution and diluted to 200 ml with distilled water.

3.5 0.05 N HCl (N/200)

4.3 ml of concentrated hydrochloric acid will be mixed with 995.7 ml distilled water.

3.6 Methyl red indicator